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IX. SPECIFIC ANAPHYLACTIC SHOCK

F. G. NOVY AND P. H. DEKRUIF

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For the study of this phenomenon, the rat was elected as first choice for the reason that previous experience had shown that it exhibited 2 very important characteristics which, though fully discussed heretofore, may be briefly mentioned.

In the first place, its serum was found to yield a most powerful anaphylatoxin. Under proper conditions, as has been shown, it is possible to render normal rat serum toxic, within 15 minutes, so that from 0.2 to 0.25 c.c. will kill a 200 gm. guinea-pig on intravenous injection. With inulin, it has been possible to reduce the lethal dose to 0.15 c.c. (Part X). When it is recalled that the primary toxicity of normal rat serum is such that it only rarely kills guinea-pigs in dose of 4 or 5 c.c., it will be seen that this means, at least, a 20 or 30 fold increase in its poisonous property. The sera of rabbit and guinea-pig, under like conditions, give at best only a threefold to sixfold increase in toxicity. It is obvious, therefore, that the rat serum is decidedly more labile than the sera mentioned, and it seemed reasonable to believe that the plasma in corpore would share this characteristic.

In the second place, it was found that the rat was insusceptible to enormous doses of anaphylatoxin. Thus, of a given anaphylatoxin which killed a 247 gm. guinea-pig in dose of 0.25 c.c., as much as 15 c.c. were injected into a 151 gm. white rat without causing death, though the shock was severe (Table 58). The rat, therefore, compared with the guinea-pig, possesses a remarkable tolerance as to this poison. At first sight, it would seem that this resistance was cellular in character, an adaptation or off-set, as it were, to the highly labile plasma of the animal. But, it is possible that the real explanation will be found elsewhere, perhaps in purely physical reactions, reversive in character. The fact remains, however, that, weight for weight, the rat will tolerate more than 100 times the amount of poison necessary to kill a guinea-pig.

In view of the foregoing acts, it would seem that in anaphylactic shock the guinea-pig may develop relatively but little poison, since the production of a single fatal dose is sufficient to cause death. At most, one could expect but a few lethal doses since a guinea-pig serum does not yield a powerful poison; it is possible, with agar, to render it toxic in 1 c.c. dose (Part II). Assuming, for example, that a 200 gm. guinea-pig has 12 c.c. plasma, it would seem that, at most, 12 lethal doses could be expected, supposing that the plasma was as toxifiable as the serum. However, it may be more reactive, owing to the presence of the labile fibrinogen.

On the other hand, in the rat because of its tolerance for the poison, an anaphylactic shock if induced should be the result of the formation of a large amount of poison, probably in excess of 100 guinea-pig lethal doses. It may be questioned whether such a large amount of poison could be produced within the rat. Thus, assuming as above that a 200 gm. rat has 12 c.c. plasma, it would be capable of yielding 80 guinea-pig lethal doses, each being 0.15 c.c. This calculation would show that unless the fibrinogen, or perhaps the cell plasma, reacted and thus increased the amount of poison, it would not be possible to produce a fatal specific anaphylactic shock in the rat. These considerations show that the blood of the rat should be better adapted than that of the guinea-pig for the study of anaphylactic reaction. The rat proved to be a most useful reagent in this study.

SPECIFIC ANAPHYLACTIC SHOCK IN THE SENSITIZED RAT

In order to test their behavior to specific shock, a large number of rats were sensitized by the intravenous injection of diverse sera and also egg-white. Rather unexpectedly, it was found that the rat did not respond, certainly not fatally, to the second injection. An examination of the literature failed to add any light, for, apparently, but a few workers have attempted to induce anaphylactic shock in rats.

The first mention of tests with this species was made by Arthus[1] who stated that he had observed anaphylactic phenomena in guinea-pigs and rats after repeated injections of horse serum, and that the results would be described later. It has not been possible to find, in his subsequent papers, any further allusion to this matter. Rosenau and Anderson[2] mention having tested rats, among other animals, but the work was to be reported on at a later date. Uhlenhuth and Haendel[3] did not succeed in producing anaphylaxis in mice and rats; and Trommsdorff,[4] repeating the work in Uhlenhuth's laboratory, sensitized rats and mice to egg-white but in neither could he induce symptoms, much less fatal anaphylactic shock. The behavior of mice to anaphylactic shock has been studied by many workers with varying results. Of these, Braun,[5] Ritz[6] and also Sarnowski[7] succeeded. It is therefore possible that given special conditions, even rats may be made to respond. According to Ritz and Sachs,[8] the mouse, while insusceptible to anaphylatoxin, reacts with peptone and to specific anaphylactic shock.

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[1] Compt. rend. Soc. de biol., 1903, 55, p. 819.

[2] Bull. No. 29, Hyg. Lab. U. S. P. H. S., 1906, p. 57.

[3] Centralbl. f. Bakteriol., I, Ref., 1910, 47, Beiheft, p. 68.

[4] Arb. a. d. K. Gsndhtsamte, 1909, 32, p. 567. Centralbl. f. Bakteriol., I, Ref., 1909, 44, Beiheft, p. 152.

[5] München. med. Wchnschr., 1909, 56, p. 1880. Ztschr. f. Immunitätsf., 1909, 4, p. 600.

[6] Ztschr. f. Immunitätsf., 1911, 9, p. 331. Centralbl. f. Bakteriol., I, Ref., 1910, 47, Beiheft, p. 63.

[7] Ztschr. f. Immunitätsf., 1913, 17, p. 577.

[8] Centralbl. f. Bakteriol., I, Ref., 1911, 50, Beiheft, p. 45.

The greater part of the tests on rats were made either with horse serum or egg-white, and the results are of sufficient interest to be given at length. Tables 122 and 123 embody the results obtained with horse serum; in the first of these tables, the rats received only a single sensitizing injection of 0.01 c.c. of the serum (1 c.c. of 1 : 100). When retested after a suitable interval, the rats showed practically no effects except when the antigen was given in large dose (Nos. 5 and 6).

Since Sarnowski's work on mouse anaphylaxis had shown that a single injection of a small amount of serum did not sensitize as well as a large amount, it seemed likely that the nonfatal, and even mild results given in Table 122, were due to a similar condition. Accordingly, sensitization, with several varying doses, was resorted to. In Table 123 will be found the results of such tests on 14 rats. It will be seen that nearly all of the test animals showed more or less respiratory disturbance, at times some dyspnea and slight jerky spasms, but at no time was a fatal result produced. As indicated in the foot-note to the table, 4 of the rats were later retested with a fourth injection with no particular increase in the symptoms.

TABLE 122

ANAPHYLACTIC REACTION IN RATS SENSITIZED WITH SINGLE SMALL DOSE OF HORSE SERUM

Rat		Serum (c.c.)		Result
No.	Weight	First Inject.	Second Inject.	
1	150	0.01	0.5 on 13th day	Nil
2	156	"	1 " " "	"
3	148	"	2 " " "	Very irregular respiration
4	158	"	1 " 52nd "	Slight respiration trouble
5	168	"	3 " " "	Severe shock; irregular respiration, slight spasms, severe dyspnea, depression
6	150	"	3 " " "	Very severe dyspnea, spasms, in 3 minutes apnea. Used for transfusion
7C	155		2	Deep rapid respiration

All injections intravenous. Nos. 4 and 5 were reinjected as Nos. 1 and 2 of Table 123. No. 7 was a normal rat and served as a control.

It will be observed that rats which received 2 or 3 preliminary injections did not react much more than those that had but one (Table 122). These results clearly show that the rat does not respond with severe anaphylactic shock, in the manner of the guinea-pig. The remarkable tolerance of the rat to anaphylatoxin, which has been pointed out heretofore, finds further corroboration in these experiments, since not enough of the poison is formed in the sensitized rat to produce death. That there is a partial reaction, there can be no doubt, judging from the symptoms; further proof will be given later on, when it will be shown that in rats thus treated there is an actual in-vivo production of poison.

The results obtained with different antigens, such as the sera of guinea-pig, rabbit, beef, and with solutions of egg-white, were essentially the same as those presented above. The incompleteness of the anaphylactic reaction when an undiluted antigen was used, as was the case in Tables 122, and 123, suggested the possibility that a better result might be obtained with one that was diluted. The basis for this belief will be given further on. Accordingly, 2 such tests were made with an antigen which was diluted nineteenfold (Table 124, Nos. 6 and 7). In the first of these, with dilute beef serum (No. 6), the result was distinctly more severe than the companion test where 4 c.c. of the undiluted serum were given, this amount being 16 times that given No. 6. In this test the serum was diluted with salt solution.

TABLE 123

ANAPHYLACTIC REACTION IN RATS SENSITIZED WITH MULTIPLE DOSES OF HORSE SERUM

Rat		Serum (c.c.)		Result
No.	Weight	Preparation	Last Injection*	
1	135	Jan. 3, 0.01; Feb. 24, 1.0	3 on 16 day	Respiration, deep, jerky
2	160	" " " " 3.0	" " " "	Severe dyspnea
3	152	" " Jan. 16, 2.0	1 " 39 "	Slow jerky respiration. No spasms
4	150	" " " " 1.0	" " " "	Short severe dyspnea
5	110	" " " " 0.5	" " " "	Slight spasms
6	165	Dec. 30, 0.01; Jan. 17, 0.5	" " 38 "	" " Deep respiration
7	190	" " " " " "	3 " " "	No spasms. Deep respiration
8	125	" " " " " "	" " " "	Some dyspnea. Deep respiration
9	150	" " " " " "	" " " "	Slightly irregular respiration
10	146	" " " " " "	" " " "	Rapid deep respiration
11	135	" " " " " "	" " " "	Nil
12	124	" " " " " "	" " " "	Nil
13	115	" " " " " "	" " " "	Deep respiration
14	123	" " " " " "	" " " "	Very deep respiration

* The time given refers to the number of days since the last sensitizing dose was given. All injections, intravenous. Nos. 3, 6, 9 and 10 were each given a fourth injection, 16 days later, of 3 c.c. Marked peripheral irritation, irregular respiration to slight dyspnea, with light jerky spasms were noted in the first 2; the effects in the last 2 were much less severe.

For the second test with diluted antigen, a rat sensitized to rabbit serum was used; the dilution was effected with distilled water instead of salt solution. It will be noted that the outcome was a protracted death, such as has been usually observed in mice. This was the first instance of fatal anaphylactic shock in the rat.

In the tests with guinea-pig serum, Nos. 1 to 3, Table 124, only a moderate respiratory disturbance was produced, corresponding to that seen in the work with horse serum. It is of interest to observe that beef serum, which is relatively toxic to a guinea-pig, produced very little effect, in the control normal rat No. 4, in dose of 4 c.c. This is in keeping with the tolerance for anaphylatoxin which the rat has been shown to possess, and it suggests the desirability of studying the behavior of the rat to various highly toxic sera, such as those of eel, man, etc. In the sensitized rat, the beef serum caused a fair shock, but a considerably greater effect followed the use of the highly diluted antigen (No. 6). The result with the similarly diluted rabbit serum (No. 7) was heuristic and opened up an interesting line of study. The egg-white being more alien to an animal than a serum could be expected to give a greater reaction; this, however, was not the case and the explanation is that a more concentrated solution, corresponding in protein content to a serum, would have been probably better than a 1 per cent. solution. Likewise, the use of high dilutions of egg-white with distilled water, similar to the dose in Nos. 6 and 7, will be found desirable (Table 125).

TABLE 124

ANAPHYLACTIC REACTION IN RATS SENSITIZED WITH DIFFERENT ANTIGENS

Antigen	Rat		Antigen (c.c.) Given on		Result
	No.	Weight	First Inject.	Second Inject.	
Guinea-pig Serum	1	145	0.25	1 on 17 day	Some dyspnea. Very deep respiration
	2	125	"	2 " " "	Very irregular deep respiration
	3	140	"	3 " " "	" " respiration
Beef serum	4 C	150		4	Respiration shallow, interrupted
	5	140	0.25	" on 16 day	Fair shock. In 10 min. severe peripheral irritation. Resp. irregular
	6	105	"	5 " " " *	Severe shock. Excited. Deep resp.
Rabbit Serum	7	127	"	5 " " " †	2 hrs. 15 min. Excited. Very rapid respiration, then slow and jerky
Egg-white‡	8	120	1	1 " " "	Very excited. Very rapid respiration, later slow and deep
	9	125	"	5 " " "	Slow, labored respiration

* 0.25 c.c. beef serum diluted with 4.75 c.c. salt solution.

† 0.25 c.c. rabbit serum diluted with 4.75 c.c. distilled water.

‡ This was a 1% solution of Kahlbaum's desiccated egg-white, dissolved in 0.85% salt. All injections were intravenous.

NONSPECIFIC ANAPHYLACTIC SHOCK IN SENSITIZED RATS

The foregoing experiments show quite conclusively that the antigen, when injected by itself into a sensitized rat, is incapable of releasing a fatal shock under conditions which would have met with a prompt response in a guinea-pig. Some indication of a reaction, however, was in evidence and this led to the belief that a slight alteration in the state of the blood plasma might yield a better result. A basis for such view is to be found in the known fact that undiluted sera often fail to give specific reactions, whereas when diluted they are instantly effective (Neisser and Wechsberg[9]).

In particular, we were guided by an unpublished observation made some years ago by one of us (Novy) concerning the paradoxical action of hyperimmune rat blood on relapsing spirochetes. This serum was tested undiluted, as well as in serial dilution, up to as high as 1 : 1000, for its agglutination, spirillicidal, and lytic reactions. The action of the highly diluted serum was immediate and striking; the spirochetes on contact with such dilutions instantly showed intense hyperexcitability, then rapidly agglutinated in enormous stellate masses, after which the motion soon decreased, then disappeared; the now motionless spirochetes each developed one or more globular swellings (plasmoptysic in character and not buds as supposed by some); this process rapidly went on to full lysis so that in a few minutes nothing but a mass of minute granules was left. In brief, the diluted hyperimmune serum induced an intense in-vitro Pfeiffer's phenomenon.

In very low dilutions, especially in undiluted serum, the reaction was different. At first, there was hyperexcitability, then moderate agglutination, accompanied by a gradual decrease in motility up to a certain point; then, of a sudden, the unagglutinated cells began to show a quickening motion, and this revival extended to all of the organisms, the spirochetes disengaged themselves from the loosely agglutinated masses and became free and active just as if it were a normal serum. This paradoxical behavior of the diluted, versus the undiluted serum is connected with a further observation where rats immunized by 30 or more injections of spirillar blood, showed active spirochetes in their circulation, almost continuously during the hyperimmune stage. Pfeiffer's isolation of the live cholera vibrio from the peritoneal cavity of the hyperimmune guinea-pig is of like meaning.

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[9] München. med. Wchnschr., 1901, 48, p. 697.

Both reactions are obviously of the Neisser-Wechsberg type; they show clearly that while antibodies are present in such immune blood or serum, they are unable to act either on account of the aggregation or concentration of the constituents, or because of some hindering or calyptic substance. Parenthetically, it may be added that the cause of "fastness" in an organism may be sought in the reciprocal aggregation of its protoplasmic colloids to a disturbed state in the host. Bearing in mind the view expressed, experiments were planned to test the action of highly diluted antigen upon the sensitized rat. The first 2 tests of this kind, given in Table 124, led to further trials with large amounts of fluid, which will now be considered.

TABLE 125

THE EFFECT OF DILUTE ANTIGEN (1: 14) ON RATS SENSITIZED TO EGG-WHITE

Rat		Antigen (c.c.) Given on			Result
No.	Weight	First Inject. (undil.)	Second Inject. (diluted 1:14)	Dose per 100 gm.	
1	215	1	15 on 12 day	7.0	Very rapid fluttering respiration, later, interrupted and deep. Muscles leg twitch. No spasms
2	205	"	" " 17 "	7.3	Apnea while injecting. Slow return of respiration. Dyspnea. Muscle twitch
3	154	"	" " " "	9.8	Same as No. 2
4	168	"	" " " "	8.9	2′30″. Apnea while injecting. No return of respiration, muscle twitch
5	198	"	" " " "	7.6	Same as No. 2*
6	185	"	" " 18 "	8.1	Immediate respiratory trouble. Dyspnea*
7	150	"	" " " "	10	Respiration stops while injecting*

The time required to inject 15 c.c. ranged from ¾ to 1¼ minute.

* The rats thus designated were bled for transfusion experiments in Table 134. All injections were intravenous.

Rats sensitized to egg-white. — For the experiments given in Table 125, the antigen, which was a 1% solution of egg-white in salt solution, was diluted with 14 parts of distilled water, and the test dose of this mixture was 15 c.c. The result was marked for, even while injecting, the respiration became fluttering and ceased entirely; in some it returned, in others it did not. Rapid muscular twitching of the injected leg, especially about the point of injection, was nearly always observed; this may be interpreted as a local cellular anaphylactic response to the dilute antigen.

The rats were sensitized by an intravenous injection of 1 c.c. of the 1% egg-white solution and were used 12 to 18 days later. Three of the rats mentioned in Table 125 were opened, while in shock, and blood was drawn from the heart and injected into guinea-pigs for the purpose of demonstrating the in-vivo production of poison (Table 134); in all of these animals, the characteristic anaphylactic findings were noted: maximal lung distention, and drop in blood pressure. The heart of No. 2, examined within 3 minutes after the injection, was found to contain clot and much fluid blood; a similar condition was noted in No. 4; in No. 5 there was no sign of clot in the heart, even 6 minutes after death; in No. 7, notwithstanding the large volume injected, the drop in blood pressure was such that 3 c.c. of blood could be drawn with difficulty; in the heart of No. 6, when examined 5 minutes after exposure, clot was found, but this was clearly postmortem, since the blood drawn for transfusion, at once after injection, was perfectly fluid. This tendency to form clot in the heart of the sensitized animal shows a highly labilized condition of the plasma proteins, which will be brought out again in connection with Table 127.

TABLE 126

The Effect on Normal Rats of Dilute Egg-White (1: 14), A; Distilled Water, B

Exper.	Rat		Fluid Injected		Result
	No.	Weight	Amount (c.c.)	Dose per 100 gm.	
A Egg-White	1	145	15	10.3	Deep respiration, otherwise nil
	2	165	10	6.0	Same
	3	159	15	9.4	"
	4	206	"	7.3	"
	5	186	"	8.0	" *
	6	210	"	7.1	" *
B Distilled Water	7	162	"	9.3	"
	8	158	12	7.6	"
	9	160	12.1	7.5	"
	10	200	15	"	"
	11	175	13.1	"	"

* The rats thus designated were bled for transfusion experiments in Table 134. The time taken for injections was ¾ - 1¼ min.

The severe results obtained in the foregoing experiments raised the question as to whether they were not due to the mere injection of so large an amount of fluid. Controls were, therefore, made at once with normal rats. Two series of control tests are given in Table 126; of these, Series A was made with the very dilute egg-white, the same as that used in Table 125, while Series B was made with plain distilled water.

In regard to Series A, the normal rat shows an entirely different behavior from that of the sensitized one; practically no effect followed the injection of these amounts of diluted antigen. These tests, it may be added, were made on the same day as those of Table 125.

As a further control, normal rats were injected with distilled water (Series B) ; the results of these tests are also to be found in Table 126. It is to be noted that distilled water may be injected intravenously in large dose without effect.

Since the normal rat was found to be practically unaffected by large doses of diluted antigen, or distilled water, it was necessary as a further control, in order to establish the specific nature of the shock observed in sensitized rats, to test the effect of a large volume of distilled water in such rats. This experiment led to a surprising result. It revealed the fact that the shock results seen after the injection of the diluted antigen were not due to the latter but in reality were due to the volume of water used. In other words, the sensitized rat, unlike the normal one, responds to the injection of distilled water with a shock which can only be interpreted as anaphylactic. The reaction is considered, therefore, to be a nonspecific anaphylaxis, since the antigen is not necessary to discharge the shock in a sensitized rat.

TABLE 127

THE EFFECT OF DISTILLED WATER (A), AND OF SALT SOLUTION (B), ON RATS SENSITIZED TO EGG-WHITE

Exper.	Rat		Fluid Injected		Result
	No.	Weight	Amount (c.c.)	Dose per 100 gm.	
A Distilled Water	1	163	12.2	7.5	Dyspnea and apnea while injecting. Muscle twitching. No recovery
	2	175	13.1	"	Same as No. 1
	3	160	12.1	"	" " "
	4	175	8.75	5.0	Very near-kill. Dyspnea, convulsions thrown, recovers in 10 min.
	5	198	10	"	5′. Apnea while injecting. Convulsive jerks. Muscle twitching
	6	195	"	"	Practically nil
	7	175	8.75	"	Nil
B Salt Solution	8	210	15.75	7.5	Practically nil
	9	196	14.7	"	" "
	10	140	10.5	"	Some respiratory trouble toward end injection. Depressed. Few jerky spasms

All injections were intravenous. The injection time was usually from 50 to 60 seconds.

The results of experiments in which varying amounts of distilled water were injected into sensitized rats are given. in Table 127. The rats were all sensitized by the intravenous injection of 1 c.c. of the 1% egg-white solution, and were tested on the 15th and 16th days. The controls for this set of tests are to be found in Table 126, Series B.

Autopsy, made about 3 minutes after the injection, showed in Nos. 1, 2, 3, and 5 partial lung distention and clot in the heart. Clotting was also observed in connection with tests of Table 125.

The results presented in Table 127 show that rats sensitized to egg-white are almost instantly killed by the injection of distilled water, when given in dose of 7.5 c.c. per 100 gm. body-weight; 5 c.c. per 100 gm. is less certain in its effects and may even be nil. The individual variation of the rat must be considered. On the other hand, distilled water in dose of 7.5 gm. or even more per 100 gm. of normal rat is innocuous (Table 126). These results are paralleled by those obtained with rats sensitized to horse serum.

Extremely interesting are the results obtained in tests Nos. 8 to 10 of Table 127 where the rats were sensitized in the same way as the others, and retested at the same time with salt solution (0.85%). By contrast with Nos. 1 to 3, the salt solution was practically harmless, whereas the distilled water tests were fatal. Some effect was obtained in No. 10, but this may have been due in part to the fact that the animal was rather weak, if not sickly when injected.

It may also be noticed that the results given in Table 127 are decidedly more severe than those in Table 125 where sensitized rats were given the same volume of the diluted egg-white. At first, it might seem that this difference was due to the small amount of salt injected with the egg-white. It is more likely, however, that the observed difference is due to the experimental conditions. In the tests of Table 125, the rats were etherized, fixed, and then allowed to recover, after which they were injected, while those of Table 127 were injected directly.

It is evident that the sensitized rat is different from the normal one. Though it does not respond like the sensitized guinea-pig to an antigen, nevertheless, the physical state of its plasma proteins must be greatly altered. It no longer possesses the resistance or tolerance of the normal rat, and the mere injection of distilled water, it may be assumed, produces an intense disruption of the colloidal equilibrium, resulting in intramolecular changes which yield on the one hand, anaphylatoxin, and on the other, increased rapidity of coagulation, or fibrin. The immediate cause of death must be sought in the production of anaphylatoxin through tautomeric modification of a matrix present in the plasma, if not in the cytoplasm. The rapid formation of clot in the blood of the shocked animal is not the cause of death since the clot is not present if the heart is examined at once after death. A somewhat similar condition arises when a large dose of rabbit serum is injected into a guinea-pig. As shown in Part VIII, small doses of this serum may kill without any clot appearing in the heart, whereas with large doses, coagulation is observed, at autopsy.

It has been pointed out on page 777 that a rate of 200 gm. with 12 c.c. of plasma, is capable of developing from 50 to 80 guinea-pig lethal doses, and since it has a natural tolerance for 100 of such doses, the fatal results obtained are rather paradoxical. The first explanation for this discrepancy is that the tissue cells of the sensitized rat have become more susceptible and reactive. If that were so, a sensitized rat should reveal such increased susceptibility when injected with a large multiple dose of the poison. To test this point, a rat serum was toxified by the sol-gel method so that 0.25 c.c. was the lethal dose for a 180 gm. guinea-pig. Of this anaphylatoxin, 10 c.c., representing 40 lethal doses, were injected intravenously, in 55 seconds, into a sensitized rat of 187 gm.; only a slight respiratory disturbance and depression followed. A second sensitized rat (170 gm.) was then given a mixture consisting of 7.5 c.c. of this anaphylatoxin and 8.2 c.c. of distilled water (5 per 100), to see if the presence of distilled water would aid the poison; the result in this case was perhaps a trifle more severe than in the former.

These 2 tests show that the action of the distilled water in the sensitized rat is not limited to the toxification of the plasma, but that the explosive reaction probably involves the sensitized cell plasma as well. A sensitized animal does not possess an increased susceptibility to anaphylatoxin; it can, however, produce poison more readily. Sensitization may mean a change in the cytoplasm as well as in the blood plasma.

Rats Sensitized to Horse Serum.—The results obtained with rats sensitized to horse serum correspond to those noted in connection with egg-white (Table 125). Though the tests were not as extensive as with the latter, they sufficed to show that the sensitized rat responds quickly to the injection of either dilute antigen or distilled water, whereas the control normal rats (Nos. 11 to 13) which received the same amount of the dilute antigen showed practically no effect. The controls for the action of distilled water upon normal rats are to be found in Table 126.

The rats employed for tests Nos. 1 to 6 of Table 128 were sensitized by a single intravenous injection of 0.25 c.c. of horse serum; Nos. 4 and 5 were tested 12 days later, the others on the 20th day. Nos. 7 to 10 had previously received multiple injections of the antigen (Table 123), and were retested 30 days after the last injection, with varying concentrations of horse serum. The antigen concentration is indicated in the table. The injection time varied from ¾ to 1½ minute, except in case of No. 7 which took 2½ minutes; recovery was probably due to this fact.

TABLE 128

The Effect of Dilute Antigen in Rats Sensitized to Horse Serum

Exper.	Rat		Antigen			Result
	No.	Weight	Dilution	Amount (c.c.)	Dose per 100 gm.	
A	1	149	1+14	15	10	1′30″. Dyspnea, spasms, convulsions
	2	?	"	"	—	45″. Instant apnea
	3	158	"	"	9.5	2′. While injecting dyspnea, then apnea; followed by prompt return of respiration, then peripheral irritation, dyspnea, spasms, convulsions
	4	175	"	"	8.6	Marked respiratory effects. Died during night
	5	180	1+9	10	5.5	Nil
	6	?	"	"	—	Slight respiratory trouble
B	7	135	1+19	10	7.4	Respiration suppressed but returns
	8	141	"	"	7.1	1′45″. Respiration suppressed while injecting
	9	152	1+39	"	6.6	Respiration suppressed but recovers
	10	150	1+59	15	10	1′40″. Respiration suppressed. Muscle twitching
C Control	11	156	1+14	15	9.6	Nil
	12	170	"	"	8.8	"
	13	152	"	"	9.9	"

Nos. 11 to 13 are normal rats which were injected with the dilute antigen as controls.

It will be seen that the dilute antigen in dose of 7 c.c. or more per 100 gm. is usually fatal to sensitized rats. That this volume of liquid of itself is not responsible for the effects observed will be seen from the control tests Nos. 11 to 13. The effects produced are the same as those seen in connection with the tests of rats sensitized to egg-white. Autopsy usually showed permanent maximal lung distention. In the 5 acute deaths of Tables 128, the heart, examined within 3 minutes after death, showed much clot; it is possible that in one of these the change was premortal, but of that there can be no certainty.

Since the dilute antigen has a marked action upon rats sensitized to horse serum, it was of interest to see if distilled water would not have the same action. The experiments in Table 129 were made with this object in mind. The rats were sensitized by intravenous injections of 1 c.c. of horse serum, and were tested 14 days later. The time required to make these injections was about 1 minute.

It will be seen from the table that rats sensitized to horse serum, on injection with distilled water, behave the same as those prepared with egg-white. The dose of 7.5 c.c. per 100 gm. body-weight is acutely fatal to the sensitized rat; 5 c.c. per 100 gm. has less or no effect. It is significant that the injection of salt solution in dose of 7.5 c.c. per 100 gm. results in much less effect (Nos. 9 and 10). Even the addition of antigen to the salt solution is not enough to overcome the inhibitory action of the salt (Nos. 7 and 8). Similar results after salt injection into rats sensitized with egg-white are given in Table 127, B.

TABLE 129

THE EFFECT OF DISTILLED WATER ON RATS SENSITIZED TO HORSE SERUM

Exper.	Rat		Fluid Injected		Result
	No.	Weight	Amount (c.c.)	Dose per 100 gm.	
A	1	175	13.1	7.5	1′30″. Respiration suppressed while injecting. Jerky spasms. Muscle twitchings
	2	204	15	"	1′30″. Dyspnea. Convulsions
	3	150	11.2	"	1′. Apnea. Severe convulsions
	4	191	9.5	5.0	Respiration almost suppressed
	5	198	10	"	Nil
	6	180	5	2.8	"
B	7*	173	13	7.5	Respiration not suppressed, but severe depression. Died during night
	8†	175	"	"	Respiration not suppressed
C	9‡	175	"	"	Respiration suppressed, but returns very shallow. Slight spasms
Control	10‡	160	12	"	Respiration very rapid, shallow

* Received 1 c.c. horse serum + 12 c.c. salt solution.

† Received a mixture of 1 c.c. horse serum, 6 c.c. of salt solution and 6 c.c. of distilled water.

‡ These are sensitized rats which received only salt solution.

The autopsy of Nos. 1 to 3 showed partial to maximal distention of the lungs. The heart of No. 1, examined 3 minutes after death, showed a large clot; but in Nos. 2 and 3 where the examination was made in 2 and 1 minutes, respectively, after death, no clot was present. This fact, as pointed out before (Table 127), indicates clearly that coagulation is not the immediate cause of death.

NONSPECIFIC SHOCK IN SENSITIZED GUINEA-PIGS

The peculiar behavior of sensitized rats to distilled water, on injection, yielding an intense nonspecific anaphylactic shock, made it desirable to ascertain if a similar condition could be demonstrated in sensitized guinea-pigs. With this object in view, a series of tests were made, the results of which are given in Table 130. The guinea-pigs were sensitized by intravenous injection of 1 c.c. of the 1% solution of egg-white; they were tested 12 days later (Nos. 1 to 5).

TABLE 130

The Effect of Distilled Water on Guinea-Pigs Sensitized to Egg-White (A, B); also on Normal Guinea-Pigs (C)

Exper.	Guinea-Pig		Distilled Water		Injection Time	Result
	No.	Weight	Amount (c.c.)	Dose per 100 gm.		
A	1	260	13	5	1′20″	Very slight
	2	240	17	7.5	1′45″	Slight
	3	205	20	10	2′	Slight
B	4	250	12.5	5	35″	Very slight
	5	201	15	7.5	45″	32′. At once apnea, recovery. Then relapse
C Controls	6	200	10	5	1′	Nil
	7	195	14.6	7.5	1′30″	Very slight
	8	199	15	"	1′	1′40″. At once apnea
	9	203	"	7.4	1′	1′20″. " " "
	10	210	"	7.1	3′	Slight
	11	202	10	5	45″	"

It will be seen from the table that the sensitized guinea-pigs, on injection with distilled water, behave about the same as the normal animals (Nos. 6 to 11). When the speed of injection was halved, as in Exper. B., there was possibly an increased effect but, as shown in the controls (Nos. 8 and 9), this cannot be ascribed to the sensitized condition. By increasing the time of injection to 2 minutes, it was possible to inject 10 c.c. per 100 gm. without appreciable effect (No. 3). As to the speed of injection, even a fraction of a minute seemed to markedly affect the results. This was especially seen in connection with the controls where dose of 7.5 c.c. per 100 gm. was shown to be fatal, provided it was injected within 1 minute. Thus, while Nos. 8 and 9 died rapidly under these conditions, Nos. 7 and 10, with a longer injection time, exhibited little effect. The autopsies made 3 minutes after death showed maximal distention of lungs in No. 8, and partial distention in No. 9; the hearts were not beating, and clot was present in both.

The rather indifferent results obtained in these tests suggested the possibility that the guinea-pigs were not sufficiently sensitized, but No. 4 retested with 1 c.c. of egg-white, 1¾ hours after the injection of the distilled water, promptly responded with atypical shock and died in 3′30″. Again, Nos. 1 to 3 were retested, in like manner 30 hours later; No. 1 apparently showed no effect and it seemed as if desensitization had resulted from the previous injection of water; but Nos. 2 and 3 responded with typical shock, showing that the result mentioned was probably due to individual variation in resistance of the test animals.

The foregoing experiments show that the rapid injection of much distilled water is fatal to the normal guinea-pig, while the sensitized animal is not any more susceptible. By contrast, sensitized rats react more readily than normal ones, and furthermore, speed is not as important a factor. The question naturally arises as to the reason for the difference in the behavior of the sensitized rat and guinea-pig. Undoubtedly, the explanation is to be found in the relative ease with which the respective sera are toxified. Thus, under like conditions, working with an agar sol-gel, the toxic dose of the treated rat serum will be at least 6 times that of the guinea-pig, 1.5 c.c.: 0.25. The rat plasma, inherently labile, is rendered more so by the process of sensitization, whereas that of the guinea-pig is not. It will be shown later (Tables 137 and 144) that sensitized rat serum is considerably more easily toxified by the action of distilled water than is normal serum. The in-vitro action of distilled water has not been tested, in like manner, on normal and sensitized guinea-pig serum. It would be of interest to do so and also to test the lability of the normal and sensitized sera as to speed of poison-production on treatment with agar sol-gel, or with inulin.

It has been pointed out in connection with Tables 127 and 129 that salt solution, in amounts corresponding to the fatal dose of distilled water, produces but moderate effects in rats. This fact also holds true for guinea-pigs, as will be seen from the results of tests given in

Table 131. The first 10 tests were made with the intent to bring out, if possible, a nonspecific shock with a dilute alien protein in normal guinea-pigs; this result was not realized, though with larger amounts of such protein it must be obtainable.

Of the 3 tests (Exper. A) made with egg-white, diluted with distilled water, one resulted in acute death, one in a fair shock and one showed very little effect. The symptoms were the same as those following the injection of distilled water (Table 130); the autopsy of No. 1, made 3 minutes after death, showed maximal distention of lungs and clot in the heart.

TABLE 131

Effect on Normal Guinea-Pigs of Salt Solution with Egg-White (B); and Without (C); A Received Egg-White Plus Distilled Water

Exper.	Guinea-Pig		Fluid Injected		Injection Time	Result
	No	Weight	Amount (c.c.)	Dose per 100 gm.		
A*	1	210	15	7.2	1′	2′. At once apnea
	2	200	"	7.5	"	Fair
	3	201	"	"	"	Very slight
B†	4	195	"	7.7	"	Practically nil
	5	199	"	7.5	"	Slight
	6	226	"	6.6	"	"
	7	210	"	7.1	1′15″	1 hr. 20 min. At once apnea, recovery, then relapse
	8	218	"	6.9	1′	Slight
	9	230	"	6.5	"	Very slight
	10	233	10	4.3	45″	" "
C‡	11	210	20	9.5	1′15″	Died during night. Severe, marked hypothermia, 25 C.
	12	206	"	9.7	"	Moderate
	13	204	"	9.8	"	"
	14	200?	"	?	1′30″	Slight
	15	" ?	"	?	"	Very slight
	16	" ?	"	?	45″	" "
	17	" ?	"	?	2′15″	" "

* Nos. 1, 2, and 3 received a mixture of 1 c.c. of egg-white and 14 c.c. of distilled water.

† Nos. 4 to 10 received a mixture of egg-white and salt solution: this was 2 + 8 in case of No. 10; 0.5 + 14.5 in case of No. 9; and 1 + 14 in Nos. 4 to 8.

‡ Nos. 11 to 17 were given only 0.85% salt solution; this was 37 C. in Nos. 15 to 17, and at 0 C. for Nos. 11 to 14.

The weights of Nos. 14 to 17 were not available.

In Exper. B, where the egg-white was diluted with salt solution, the results were markedly less severe; only one death occurred, and that after 1½ hours. Considering the dose per 100 gm. given in these tests, it is evident that the salt solution does not cause a reaction or disturbance in the blood as does distilled water.

In Exper. C, normal guinea-pigs were injected with salt solution alone, in amount corresponding to nearly 10% of the body-weight. In Nos. 11 to 14, the solution was iced before injection, while for Nos. 15 to 17, it was warmed to 37 C. The iced solution seemed to cause more pronounced response than that which was warmed to 37 C. This may have been, however, a matter of chance, since Nos. 14 and 15, which were made side by side, showed no difference. Similarly, varying the speed of the injection of the warm solution (Nos. 16 and 17) was without effect.

The guinea-pig, therefore, can tolerate salt solution much better than distilled water. It is well known that salt has an antagonistic action as regards anaphylactic shock, the action of anaphylatoxin, and peptone (Table 73).

The effects observed in normal guinea-pigs as a result of the injection of distilled water, or salt solution, are to be interpreted as of the same order as those seen in similarly treated sensitized guinea-pigs, especially in sensitized rats. The change or disturbance created in the colloids of the plasma is to be compared with the reaction induced by injections of agar, peptone, bacteria, etc., that is, it is anaphylatoxic.

THE IN-VIVO PRODUCTION OF POISON

The shock effects observed in a sensitized guinea-pig consequent on the second injection of the so-called antigen is clearly the result of a poisonous action. It may be assumed that a poison, in the sense of a chemical entity rather than as a property of a dispersed state, is produced in the reaction which follows the administration of the second dose. But it is a striking fact that such efforts as have been made to demonstrate the presence of a poison in the blood of a shocked animal have yielded little or no result. It would seem that considering the violence of the reaction, it should be possible to show that the blood was poisonous. This important result, we have been able to accomplish. As far as we know, the experiments to be related will present the first experimental proof of the in-vivo production, during anaphylactic shock, of an acutely fatal poison. This fact, taken in connection with the subsequent demonstration of the in-vitro production of the same poison, serves to throw new light upon the nature of the anaphylaxis.

It is reasonable to expect that if the poison is a chemical entity, that once made, it will persist in the body for some time. This, indeed, is the basis of toxicologic research concerning foreign chemical poisons. On the other hand, it is possible to conceive that the enormous mass of body colloids may exert a reversive action. This, perhaps, may explain why the recovery from acute anaphylactic shock is usually rapid and complete. The blood drawn from an animal at this stage of the shock is bound to be innocuous in the case of the guinea-pig. This makes clear, in part, the failure of previous workers to demonstrate the presence of an acute poison. A further reason lies in the fact that sensitized guinea-pigs, shocked with horse serum, yield less poison, in corpore, than those treated with the strictly alien egg-white.

Guinea-Pigs Sensitized to Egg-White.—The foregoing considerations were first applied to shocked guinea-pigs, and later to rats. The former were selected at the outset, with the expectation that they would yield enough blood for 2 or 3 consecutive transfusions, and thus make it possible to show that, in a given animal, the blood was initially nontoxic, then rapidly acquired the poisonous property, and, perhaps, again lost it. This, however, was not as feasible as it would appear, owing to the fact that the rapid fall in blood pressure, in the shocked animal, limits the amount of blood which can be drawn. This difficulty may, in part, be avoided by using sensitized guinea-pigs of maximal weight. Undoubtedly, large rabbits, or even dogs, could be used to advantage for such serial transfusions but, in these animals, the rapid disappearance of the poison cannot be expected because of their greater tolerance.

For the experiment proper, the sensitized animal and one or two recipients were immobilized at the same time, on Latapie holders; the jugulars were then exposed and covered with wet cotton compress until the moment of injection. The sensitized animal was then given an intravenous injection of the antigen, in this case 2 c.c. of egg-white. The heart was at once exposed and blood drawn up into a syringe, in such amount as desired; this was then immediately injected into the first of the recipients. After an interval of from ½ to 1 or more minutes, blood was again drawn and transferred to the second recipient.

Occasionally, some delay in drawing the blood from the heart is experienced; this may be due to plugging of the needle, but more often it is caused by the drop in blood pressure. At no time, in the following experiments, was the blood in the syringe long enough to develop primary toxicity. As shown in Table 117, guinea-pig blood may become toxic in 5 c.c. dose, when kept in the syringe for 3 minutes. Even 6 c.c. of blood can be transfused without ill effects, provided the transfer time is kept short (Table 67).

The guinea-pigs employed in the experiments of Table 132, as donors, were sensitized by an intravenous injection of 1 c.c. of egg-white, and were tested on the 23d and 24th day with 2 c.c. of this solution. The table presents the results of 4 experiments, in each of which the donor served for 2 transfusion tests. The transfer time of the second portion was always longer than that of the first, because of the drop in blood pressure; for the same reason, the amount of blood withdrawn was less than that for the first test.

TABLE 132

THE IN-VIVO PRODUCTION OF ANAPHYLATOXIN IN SENSITIZED GUINEA-PIGS SHOCKED WITH EGG-WHITE

Exper.	Test No.	Weight of		Blood Trans. (c.c.)	Total Time*	Transfer Time†	Result
		Donor	Recipient				
A	1	585	206	7.0	2′40″		Practically nil
	1a	"	208	3.0	4′55″		4′55″. Typical shock and autopsy
B	2	710	210	10.0	2′ 5″	1′45″	Very slight
	2a	"	206	3.0	5′55″	2′25″	Moderate. In 7′ temperature, 33.2 C.
C	3	524	180	5.0	2′40″	1′40″	Moderate
	3a	"	191	2.0	6′ 3″	2′30″	3′. Typical shock
D	4	475	186	5.0	2′40″	1′40″	Slight
	4a	"	185	3.0	5′28″	2′30″	5′17″

* Total time is that from start of injection of antigen until the end of injection of the recipient.

† Transfer time means the interval from the entrance of the syringe into the heart of the donor until its withdrawal from the recipient. It represents, therefore, the maximal time any portion of the blood was in the syringe. This transfer time must be kept as short as possible, otherwise in-vitro toxification may occur. The difference between the 2 times represents the reaction period within the donor.

The table shows that the immediate transfusion of blood, within the first 2½ minutes following the injection of the antigen, is practically without effect even when from 5 to 10 c.c. of blood are transferred. By contrast, the second transfusion of only 2 or 3 c.c. of blood gave 3 deaths and 1 moderate shock. The "total time" of such toxic blood was from 5 to 6 minutes, but the actual reaction time, in corpore, was about 3 minutes, whereas in the first transfusions it was but 1 minute.

The failure to kill in test No. 2a corresponds to like failures in transfusions, after injections of agar and peptone (See Charts 9 and

11). A similar drop in toxicity will be seen in Exper. C., Table 133. It is likely that this apparent decrease in toxicity is due to the test animal being more resistant than the average.

The autopsy findings were those of anaphylaxis. When examined 3 minutes after death, 2 showed maximal distention of lungs, vigorous heart beat, and blood perfectly fluid with no sign of clot; while 1 (No. 3a) had slightly collapsed lungs; otherwise the condition was the same as in the others. The symptoms were not those of the extreme convulsive, but rather of the intense quiet type often produced by the injection of toxic sera.

The first transfusions, made within about 2½ minutes after the end of the injection, were practically without effect, although at this period, the shocked donor would ordinarily be in violent convulsions, indicating that at least 1 lethal dose of poison had formed in the total blood of the animal. Assuming that the blood constitutes 10% of the body-weight, this amount of poison would be distributed in from 47 to 71 c.c. of blood. The amount of blood carried over in the first transfusion from each donor was 5-7-10 c.c.; as these amounts produced but little effect, it is reasonable to conclude that the donor, at this point, had more than 1 and less than from 7 to 10 lethal doses of poison.

The fact that the second transfusions, made about 5 to 6 minutes after the injection of the antigen, were fatal in dose of from 2 to 3 c.c. would indicate that there had been a considerable increase in the amount of the poison, this increase probably continuing for some time even after apparent death. On the foregoing assumption regarding the amount of blood present, and allowing for the first transfers, it follows that donors A, C, and D had at this time 17, 23 and 14 lethal doses, respectively.

The conclusion to be drawn from these tests, therefore, is that a poison is present in the blood of the shocked guinea-pig, at about the time it would have died from the effects of shock. The symptoms and autopsy findings resulting from such transfusions are those of anaphylatoxin.

Guinea-Pigs Sensitized to Horse Serum. — The transfusion tests from sensitized guinea-pigs, after shock with egg-white, were followed by similar attempts with horse serum, the results of which are given in Table 133. The donors in these experiments were sensitized by intravenous injection of 1 c.c. of horse serum, from 23 to 25 days before the reinjection. This second injection consisted of a like amount of serum, except in Exper. C, where the test dose was 2 c.c.; the severe effect of test No. 3 may possibly have been due to the use of this large dose, though individual susceptibility must be considered, and is indicated in the behavior of No. 3a.

These experiments showed that the guinea-pigs which were sensitized to horse serum were not as responsive to poison production as were those of the egg-white set (Table 132). After the failure to secure fatal effects in the transfusion tests (Expers. A to D), it was realized that this merely confirmed previous observations that animals which were sensitized to a strictly alien protein, such as egg-white, were more reactive than those treated with diverse sera, a fact which is especially in evidence when in-vitro experiments are made (Tables 135 and 143). While it is likely that guinea-pigs sensitized to egg-white, as pointed out above, may yield, in-vitro, from 23 to 17 lethal doses of poison, it could be assumed that those treated with horse serum gave rise to enough poison to kill the donor, not much more, perhaps only from 3 to 5 lethal doses. To detect the presence of the poison in the guinea-pig shocked with horse serum, it would require, therefore, the transfusion of a large amount of blood. With this consideration in mind, the next experiments were planned so as to increase the reaction time, and to make only one transfusion in order to get the desired large quantity of blood.

Unfortunately, the donors which were used in the subsequent experiments (E to I), were not very large; hence, it was difficult to draw much blood. This difficulty was considerable when the attempt to draw the heart blood was made after the lungs had reached maximal distention, as a result of the shock; it was this complication which, in part, caused a delay of about 3 minutes in the transfer time of No. 5. This trouble was avoided by opening up the thorax immediately after the injection of the antigen, the partial distention of the lungs interfering much less with the drawing up of the heart blood than the maximal distention. There still remained a serious obstacle which was not overcome: The great drop in blood pressure made it impossible to remove the desired amount of blood in less than 3 or 4 minutes. This accounts for the long transfer time in Expers. E to I, and the results must be interpreted with caution in view of this delayed transfusion.

It has been shown in Table 117, that 5 c.c. of normal guinea-pig blood, when held in the syringe for 3 minutes, may become fatally toxic to the homologous animal. In the experiments under consideration, only a portion of the blood was in the syringe during the entire transfer time and as a further offset to the possible criticism that the poisonous results obtained in Nos. 5, 6, 7, and 9 were due to primary toxicity developed by this extravascular delay, it should be pointed out that shocked blood does not coagulate as rapidly as does normal blood; hence the toxic effects observed are more likely to be due to poison produced by the shock than to precoagulation changes.

TABLE 133

The In-Vivo Production of Anaphylatoxin in Sensitized Guinea-Pigs Shocked with Horse Serum

Exper.	Test No.	Weight of		Blood Trans. (c.c.)	Total Time	Transfer Time	Result
		Donor	Recipient				
A	1	550	210	5	3′ 3″	2′13″	Very near-kill
B	2	650	214	10	2′18″	1′30″	Practically nil
	2a	"	213	4	3′33″		Good shock
C	3	571	205	5	1′40″	30″	" "
	3a	"	195	"	3′45″		Very slight
	3b	"	183	3	7′ 3″		Fair shock
D	4	420	203	5	2′20″	1′10″	Moderate shock
	4a	"	191	2	5′15″		Slight shock
E	5	405	180	4	6′15″	3′ ?	6′20″
F	6	405	179	7	4′52″	3′ 7″	Near-kill
G	7	380	208	8	5′35″	3′55″	5′55″
H	8	360	198	8.5	4′50″	3′10″	Slight
I	9	435	204	9.5	6′30″	4′50″	2′30″

The blood remaining in the heart of the donors, after removal of the test portion, continued to be fluid for some time. Thus, clot was absent, and the blood in the heart was fluid in donor No. 3 when examined 13 minutes after injection; in No. 5, at 15 minutes; in No. 6, at 11 minutes; in No. 7, at 15 minutes; in No. 8, at 14 minutes. On the other hand, No. 9 showed a very slight clot at 14 minutes, a condition probably brought about by the repeated injury caused by the needle in being moved back and forth from the heart into the blood vessels. The donors of Table 132, it may be added, showed a similar delayed coagulation of the intracardial blood; Nos. 1, 2, and 4 had no sign of clot when examined at 15, 11, and 12 minutes after injection, whereas No. 3, at 15 minutes, showed a very small clot.

The autopsy findings in recipient No. 5 were typical: maximal distention of lungs, vigorous heart beat, and no clot; in No. 7, the lungs showed partial distention, but the heart beat was normal and the blood, fluid. In No. 9, however, while showing maximal distention of lungs and a good heart beat, there was a slight intracardial clot; this, however, is a condition to be expected whenever large amounts of blood or serum are injected into a guinea-pig. The relation of dose to clot effects was particularly brought out in connection with Tables 103 and 104.

In considering the results presented in Table 133, it is reasonable to conclude that they show the presence of anaphylatoxin in the blood of guinea-pigs shocked with horse serum. They confirm the results obtained with egg-white. The amount of poison was distinctly less, since a like calculation applied to Nos. 5, 7, and 9 indicates the presence of but from 5 to 10 lethal doses.

Rats Sensitized to Egg-White and to Horse Serum.—Having shown that a poison was produced, in vivo, during the anaphylactic shock of sensitized guinea-pigs, it was of interest to learn whether this was equally true for the shock observed in sensitized rats. It will be recalled that the treated rat is rather tolerant to a second injection of antigen (Tables 122 to 124), and that more pronounced results were obtained when the highly diluted antigen, or plain distilled water was injected (Tables 125, 127). Hence, for the tests given in Table 134, diluted antigen was used, except in case of Nos. 5 and 6. It would have been interesting to have made like transfusions from sensitized rats after the injection of distilled water but this was not done.

For tests Nos. 1 to 4, the rats were sensitized by intravenous injection of 1 c.c. of egg-white solution and were retested on the 18th day with a mixture consisting of 1 c.c. of the antigen and 14 c.c. of distilled water. The preliminary test, No. 1, indicated the apparent need of increasing the amount of blood transferred and decreasing the reaction time. The next experiment (No. 2), made with this object in view, seemed to be entirely successful, the reaction period being but 1′40″. In test No. 3, the conditions were much the same but the amount of blood transfused was only 3 c.c., and a delayed death was obtained. Without doubt, a larger dose would have given an acutely fatal shock.

In test No. 4, the transfusion of 5 c.c. had but a very slight effect; reaction time in this instance was about the same as in Nos. 2 and 3, and the very different result cannot be ascribed to the shorter transfer time. This rat was desensitized an hour before use, by being given an intravenous injection of dilute egg-white; as a result of this treatment, the blood did not become toxic on subsequent reinjection with dilute antigen. The desensitized rat, therefore, behaved essentially the same as the 2 controls, Nos. 5 and 6. These controls were normal rats which received injections of dilute antigen, in the same amount (15 c.c.) as the sensitized rats (Nos. 1 to 4). This injection produced no suppression of respiration, as it did in the sensitized rats, and the blood transfusion likewise showed absence of poison.

TABLE 134

The In-Vivo Production of Anaphylatoxin in Sensitized Rats Shocked with Egg-White (A), and with Horse Serum (C)

Exper.	Test No.	Weight of		Antigen (c.c.)*	Blood Trans. (c.c.)	Total Time	Transfer Time	Result —
		Donor	Recipient					
A Egg-White	1	198	174	15	2	5′45″	24″	Slight
	2	185	201	"	5	2′23″	43″	4′30″
	3	150	212	"	3	2′35″	1′	6 hrs.
	4	117	198	"	5	2′	28″	Very slight
B Controls	5	186	203	"	4.5	2′45″	35″	Nil
	6	210	206	"	5	3′ 5″	1′ 5″	"
C Horse Serum	7	195	175	2	2	7′	30″	Very slight
	8	150	192	3	2	5′13″	25″	Good
	9	165	197	15	3	3′42″	1′15″	Slight
	10	123	181	"	5	2′25″	40″	Good

* Nos. 7 and 8 received the undiluted horse serum; all others were given 15 c.c. of a mixture of 1 c.c. of antigen and 14 c.c. of distilled water.

The controls in Exper. B were given the dilute egg-white solution; they were normal rats, whereas all others were sensitized.

For further controls showing the effect of transfusion of whole blood from normal rats, it will be well to consult Table 66. It will be noted therein that 4 c.c. of such blood can be transfused without appreciable effect, provided the transfer time is not much over 45 or 48 seconds; when it reaches 1 minute, this dose may cause acute death as a result of precoagulation toxicity. While this fact might raise some doubt as to the interpretation of the results in tests Nos. 2 and 3, it is to be remembered that the blood of these rats was greatly diluted by the injection of 15 c.c. of fluid and, hence, the transfer time for whole blood and that of such diluted blood are not directly comparable. Indeed, Control No. 6 shows that the transfer time of 65 seconds does not result in toxifying 5 c.c. of such diluted blood.

There can be no doubt, in view of these controls, that in the rat sensitized to egg-white, and subsequently shocked, the blood acquires almost at once a demonstrable poisonous property, which seemingly disappears in a few minutes (No. 1).

For tests Nos. 7 to 10, the rats were sensitized with horse serum. Nos. 9 and 10 were given 0.25 c.c. intravenously, and were retested on the 20th day with dilute antigen; No. 8 had received an injection of 0.01 c.c. and was tested on the 52nd day; while No. 7 received 2 previous injections of 0.01 and 0.5 c.c., respectively, the last being given 37 days before being used for this experiment. It will be seen that the transfusions from Nos. 8 and 10 resulted in the production of good shocks, indicating that some poison had formed in consequence of the injection of the antigen. The amount, however, was not enough to bring about a fatal shock. It has been shown, in connection with Table 133, that guinea-pigs, sensitized to horse serum, when shocked do not give rise to poison as readily, or in the same amount, as those which have been sensitized to egg-white (Table 132). A strictly alien antigen like egg-white appears to have a greater disturbing action than a serum.

The injection of undiluted horse serum into rats Nos. 7 and 8 caused severe shock effects; the hearts when examined 10 minutes later were beating vigorously and were free of clot. In the 6 sensitized rats, tested with the dilute antigen, the usual severe effects of rapid suppression of respiration were noted, and the hearts, when examined 5 minutes after the injection, were found to have more or less clot. In view of these severe effects, it is, perhaps, surprising to find that the transfused blood was not more toxic. Considering the large amount of fluid injected, the actual quantity of blood transfused was but one-half of the amount indicated; on this basis, it would appear that rat No. 2 developed only about 7 lethal doses of poison. This amount of anaphylatoxin cannot possibly account for the severe effects produced in the rat; it suggests, therefore, the desirability of further study along these lines.

THE IN-VITRO PRODUCTION OF POISON

The fact that the injection of so-called antigen, into a sensitized guinea-pig, is followed by anaphylactic shock indicates the formation, in vivo, of a poisonous property previously nonexistent, or an accentuation of such quality already present, though in small amount, in the test animal. Proof of the formation of such poison, in vivo, during shock, has been given, but this evidence, valuable as it is, would be incomplete unless supplemented by observations showing that the same reaction, at practically the same speed, can take place in the test tube. It was logical to expect this result, provided the necessary conditions could be met, and, as will be shown, this was successfully realized.

The production of a poison, causing acute anaphylactic death, by adding antigen to sensitized serum, in vitro, has been attempted by various workers, notably Weichardt,[10] Richet,[11] Rosenau and Anderson,[12] Anderson and Frost,[13] and others with little or no success. The only one who actually succeeded in producing acute anaphylactic death was Vaughan.[14] With his co-workers, he found that incubation, for 30 minutes, of a mixture of egg-white with the serum, or with the extracts of organs from a sensitized guinea-pig, yielded a poison which killed in 4 minutes; with the same mixtures, incubated for 90 minutes, the effects were less marked. Equally marked fatal results were obtained by incubating horse serum, typhoid, and cholera bacilli with the serum and extracts of homologously sensitized guinea-pigs. The failure on the part of other investigators, notably Doerr,[15] to understand the significance of these results is surprising.

Since death from anaphylactic shock usually occurs within 5 minutes, it is evident that at least 1 guinea-pig lethal dose of poison is produced, in vivo, within this time. Assuming that the plasma constitutes 6% of the body-weight, it follows that a 200 gm. guinea-pig carries 12 c.c., which amount, therefore, in shock, would contain at least one lethal dose of the poison. If actually several of such lethal doses are developed, it will be evident that experiments, in vitro, may possibly succeed with one-half, or one-fourth, or even less of this amount of serum, it being assumed that the serum is as reactive as the plasma. This condition is really necessary since the injection of large amounts (from 6 to 8 c.c.) of a normal serum is likely to cause severe shock and even death, by reason of the presence of anaphylatoxin resulting from preclot changes. It has been shown that the fatal dose of normal guinea-pig serum may be 6 c.c. (Table 118); that of the rabbit may be also 6 and even 3 c.c. (Tables 102, 103); while that of the rat may be 4 c.c. (Table 121).

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[10] Deutsch. med. Wchnschr., 1902, 28, p. 625. Centralbl. f. Bakteriol., I, Ref., 1910, 47, Beiheft, p. 36.

[11] Compt. rend. Soc. de biol., 1909, 66, p. 1005.

[12] Bull. 50, Hyg. Lab., U. S. P. H., S., 1909, p. 43.

[13] Bull. 64, Hyg. Lab., U. S. P. H. S., 1910, p. 29.

[14] Ztschr. f. Immunitätsf., 1911, 11, p. 675.

[15] Kolle and Wassermann, Handbuch der pathogenen Mikroorganismen, 1913, 2, p. 1040.

In view of this toxicity of normal sera, extreme care must be taken to avoid drawing wrong conclusions. An essential condition is the use of one and the same pooled serum for the experiment proper and for the control tests.

It will be shown that the addition of an antigen to a sensitized serum produces a poison in a very few minutes; it speeds up enormously a reaction which occurs, though at a considerably lower rate, when such antigen is added to a normal serum. The speed of the reaction is comparable to that which results when trypanosomes, bacteria, agar, peptone, inulin, etc., are added to a serum; this fact demonstrates the unity of the phenomena concerned.

Serum of Rat Sensitized to Egg-White: Action of Antigen.—The experiments in which the action of egg-white was tested on the serum of sensitized rats are given in Table 135. For Exper. A, 4 rats were bled 16 days after having been sensitized by an intravenous injection of 1 c.c. of a 1% solution of egg-white. As usual, the heart pipet method was used, and each blood was separately defibrinated by means of the rod; after centrifugation, the resulting sera were pooled (9 c.c.). The reagents, egg-white and serum, after warming for a few minutes at 37 C. in a Roux water bath, were combined in the ratio of 1:5 (1.8 + 9), thoroughly mixed, and a portion was drawn up into the syringe for immediate injection (No. 1); the balance was then returned to the water bath and tested at 4 and at 15 minutes after the mixture had been made.

No. 1, which received the mixture within 45 seconds after it had been made, responded with a slight reaction. Whether this effect was due to the action of antigen, in this short interval of time, or whether it was the effect of the primary toxicity inherent in serum, could not be determined; it is, however, unimportant. The test shows that the mixture at this early period was not particularly toxic, unless it be assumed that guinea-pig No. 1 was of the very resistant type. Had the amount of pooled serum been sufficient, it would have been well to have had control tests preceding and following the experiment; each of these controls would have received a mixture of 4 c.c. of pooled serum and 0.8 c.c. of salt solution. A control of this kind is given in connection with Exper. B (No. 6).

By contrast, test No. 2, which was made after an incubation of 4 minutes, proved fatal, showing that a marked rise in toxicity, even in that short interval had taken place. The remainder of the mixture, actually 1.4 instead of 1.2 c.c., after being heated at 37 C. for 15 minutes, was likewise fatal. The actual amount of rat serum injected was 4 and 1.2 c.c., respectively.

The experiment was repeated with like result. For the second trial, 3 rats, which had been sensitized 16 days before, were bled and each blood was defibrinated, centrifugated and the resulting sera (10 c.c.) were pooled. As before, the pooled serum was used at once for the experiment. The reagents, egg-white, pooled serum and distilled water, were placed at 37 C. for a few minutes, whereupon the mixtures B and C were made, 1.2 c.c. egg-white + 6 c.c. sensitized pooled serum, and 0.8 c.c. distilled water + 4 c.c. sensitized pooled serum, respectively, and at once returned to the water bath. Nos. 4, 5, and 6 were then injected at the time indicated in the table.

TABLE 135

Action of Egg-White on Sensitized Rat Serum, Ratio, 1: 5

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time* at 37 C.	
A	1	204	4.8	45″	Slight jerks. No dyspnea. Some posterior paralysis
	2	189	“	4′ 5″	3′35″. Typical shock and autopsy
	3	188	1.4	15′40″	4′25″. Typical shock and autopsy
B	4	187	4.8	4′12″	4′
	5	200	2.4	5′ 4″	3′15″
C Control	6	196	4.8	4′	Practically nil

* The time at 37 C. is that from moment of mixing until end of injection. The discrepancy in the total amount injected in Exper. A was due to the fact that each portion was measured by being drawn directly into the syringe.

Control test No. 6, in which the mixture C was used, showed no effect at a time when the antigen-serum mixture was fatal. In Expers. B and C, the same pooled serum was used and the only difference was that the former had antigen, while the latter had a corresponding amount of distilled water. The actual amount of serum injected into Nos. 4 and 5 was 4 and 2 c.c., respectively. Incubation for only 4 or

5 minutes sufficed to toxify this serum, and the minimal lethal dose was less than 2 c.c. On reference to Table 137, Nos. 10 and 13, it will be seen that 1 c.c. of a sensitized serum is fatal when incubated for 5 minutes with 6 and 9 parts of distilled water, respectively. Corresponding experiments with sensitized sera and antigens (horse, beef, rabbit, guinea-pig sera) will be found in Tables 143 and 145.

Action of Egg-White on Normal Rat Serum.—The 2 experiments in Table 135 indicate that sensitized serum on contact with its antigen, egg-white, promptly gives rise to a poison. Recognizing this fact, it is in order to inquire as to what happens when normal rat serum is incubated; first, without any addition; second, with egg-white; and third, with distilled water. The latter question will be considered on page 807.

As to the first point, it will be well to refer to Table 121, where, apparently, incubation of normal rat serum rendered it toxic in dose of 4 c.c. It must be understood that the results in this case are not strictly due to incubation; the same results would have been obtained had the serum been kept at room temperature. In that instance, the serum was primarily toxic, in dose employed, for the very susceptible individuals of the species tested, the guinea-pig. To ascertain the direct action of heat upon toxicity, it would be necessary to employ a smaller dose, making tests at hourly intervals over a long period; trials of this kind, with 2 c.c. as the dose, would be of interest, but the amount of pooled serum required for a long series of such tests is prohibitive. On a number of occasions, however, experiments of this kind were made, the dose being 1 c.c.; seemingly long incubation tended to toxify the sterile serum since severe shocks were obtained, and in one instance the 120-hour test proved acutely fatal. In another series, where the incubated serum was tested at intervals up to 56 hours, only moderate effects were obtained.

In the belief that incubation at 45 C. would assist the reaction, an experiment was tried in which pairs of tests were made at 1, 2, 3, and 6 hours, but the result was nil. It may be concluded, therefore, that labile rat serum does not readily become toxic in 1 c.c. dose when incubated at 37 or at 45 C.; on prolonged incubation, with consequent formation of sterile precipitate, it may show evidence of toxicity. Reference should be made to Table 142, Exper. B, in which a normal serum, kept at 45 C., was tested in 2 c.c. dose. It will be seen that some effect was obtained after incubation for 45 min. It would be interesting to test the effect of incubation on the sensitized rat serum, since this seems to be considerably more labile than the normal. A short series of this kind, with tests up to 45 minutes, will be found under Exper. A, Table 137; the results were nil.

In regard to the second query, as to what happens when normal rat serum is incubated with egg-white, the experiments given in Table 136 may be offered by way of an answer. Since the addition of agar, peptone, trypanosomes, etc., readily induces production of anaphylatoxin, it might be expected that egg-white would effect a similar reaction. The seemingly positive results, however, must be accepted with reserve, since the test dose contained 4 c.c. of serum, which amount, by itself, may be toxic, as has been shown to be the case in Table 121. The failure to make a parallel series of tests with the same pooled serum, without any addition, practically invalidates the experiment.

TABLE 136

Action of Egg-White on Normal Rat Serum, Ratio, 1: 5

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time at 37 C.	
A	1	207	4.8	45″	Very slight
	2	201	"	4′ 5″	Slight
	3	202	2.4	15′	Practically nil
	4	205	"	30′	" "
B	5	200	4.8	1′	Slight
	6	202	"	4′	Very slight
	7	194	"	15′	3′30″
	8	182	"	30′	Practically nil
	9	183	"	45′	3′40″
	10	204	"	60′	4′

The 2 experiments given in Table 136 were each made on the same day as the corresponding experiments of Table 135, and were intended as controls. As in those experiments, the egg-white and the pooled serum were warmed up separately to 37 C., and then mixed in the same ratio, 1: 5. Mixture A consisted of 2.4 c.c. of egg-white and 12 c.c. of serum; while B was made up of 4.9 c.c. of the former and 24.5 c.c. of the latter. The 2 mixtures were made 3 weeks apart. After mixing, tests were made at once and at the intervals given in the table.

Egg-white is probably not as poisonous as peptone when injected intravenously, and if so, it can hardly be expected to induce toxicity, in vitro, unless it is used in relatively large amounts. In order to toxify 2 c.c. of rat serum with peptone, it was necessary to use 25 mg. of the latter (Table 85); the amount of egg-white employed in Table 136 was but 4 mg. per 2 c.c. of serum. It will, therefore, be desirable to repeat these experiments with an increased quantity of egg-white.

Action of Distilled Water on Sensitized Rat Serum (*Egg-White*). —Previous tests have shown that a sensitized rat shows little or no effect upon a second injection of antigen. Unlike the guinea-pig, it does not respond with an anaphylactic shock, certainly not with a fatal one. It has also been shown that the injection of dilute antigen may produce a fatal shock; moreover, that a like amount of distilled water is equally fatal to a sensitized rat, but is without effect in a normal one. It is evident therefore, that the sensitized rat differs from the normal in its behavior to distilled water, in the same way that a sensitized guinea-pig differs from a normal one, as regards antigen. The sensitized rat injected with antigen, or dilute antigen, or distilled water, responds with an in vivo production of poison, though the amount produced may be insufficient to kill. Inasmuch as it has just been shown that sensitized rat serum, when mixed with antigen, almost immediately develops the poisonous property, it was in order to inquire as to the effect of distilled water on sensitized serum.

TABLE 137

ACTION OF DISTILLED WATER ON SERUM OF RAT SENSITIZED TO EGG-WHITE

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time at 37 C.	
A	1	201	1	7′	Nil
	2	200	"	15′	"
	3	184	"	30′	"
	4	206	"	45′	"
B	5	201	4	35″	Very slight
3 + 1	6	198	"	5′	Moderate
	7	206	"	15′	Fair
	8	199	"	32′	3′45″
C	9	206	7	1′	Very excited
6 + 1	10	195	"	5′	2′20″
	11	196	3.5	20′	Very severe
	12	194	"	31′	3′25″
D	13	198	10	5′	2′10″
9 + 1	14	197	"	15′	1′30″

The actual dose of serum injected was 1 c.c., except in Nos. 11 and 12, where it was 0.5 c.c.

The experiments given in Table 137 were made with that object in view. For Expers. A, B, and C, 4 rats which had been sensitized by intravenous injection of 1 c.c. of egg-white, 16 days before, were bled; the bloods were defibrinated, centrifugated and the resulting sera were pooled (11 c.c.). The pooled serum, previously warmed to 37 C., was divided into 3 portions; to portions B and C, distilled water, likewise at 37 C., was added in amounts indicated below. Portion A served as a control without addition of water.

A. 4 c.c. of pooled serum.
B. 12 c.c. distilled water + 4 c.c. pooled serum. (3 + 1).
C. 18 c.c. " " + 3 " " " . (6 + 1).
D. 18 c.c. " " + 2 " " " . (9 + 1).

Mixture D was made on the following day in order to supplement the first 3 experiments; the necessary serum was obtained from a rat which was sensitized at the same time and in the same way as those used for the other tests.

The mixtures were at once placed at 37 C., B and C being tested immediately; the other tests were made at intervals, as indicated in the table. The dose injected represents only 1 c.c. of serum, except in tests Nos. 11 and 12, where it is 0.5 c.c. The effects produced cannot be ascribed to either constituent taken by itself, but are directly due to the action of the distilled water on the sensitized serum.

The control set A, with the undiluted serum, showed no effect even after incubation for 45 minutes. In Series B, with the least amount of water, no fatal shock was obtained until after incubation for 32 minutes (No. 8). On the other hand, in Series C, with twice the amount of water, rapid toxification occurred since the mixture killed when tested after 5 minutes (No. 10). This mixture also killed in 0.5 c.c. serum equivalent (No. 12). In Series D, unfortunately no immediate test was made, but the indication is that a severe, if not fatal, shock would have been obtained had a test been made about one minute after mixing; it is also probable that this mixture would have killed in dose of 0.5 c.c. serum equivalent. ,

The results here given should be compared with those in Table 144, where distilled water acted on the serum of rat sensitized to horse serum. It will be seen that the 9 + 1 mixture in that instance killed in 0.5 and even 0.25 c.c. serum equivalent (Nos. 22 and 23).

The symptoms following the injection of these mixtures were typical of anaphylatoxin, especially when the volume injected was small, as in Nos. 8, 11, and 12; with the larger volumes, the shock was rapid and more of the quiet type. The time of death is figured from the end of the injection. The time of injection varied with the dose, being 10 to 20 seconds for 4 c.c.; 45 seconds for 7 c.c. and 1 minute for 10 c.c. The autopsies made 3 minutes after death showed the usual typical findings, though in Nos. 10, 13, and 14, where large doses were injected, a slight clot could be detected in the heart. This was entirely absent in Nos. 8 and 12. The presence of an extremely slight clot in No. 10, and its absence in No. 12, which received one-half the dose of the former, parallels the behavior of large and small doses of rabbit serum as regards production of clot (Tables 103 and 104).

These experiments demonstrate that a sensitized rat serum can be toxified by the addition of distilled water, and incubation. Furthermore, the toxicity thus developed is greater than that obtained by the action of undiluted antigen (Table 135). This fact as well as the great speed of poison-production is remarkable. The speed of reaction increases with the degree of dilution; in this respect, it corresponds to the observations made in connection with the spirillar paradox (page 782), the Neisser-Wechsberg phenomenon. A nonspecific reagent, distilled water, induces a more poisonous state, in vitro, as well as in vivo, than does specific antigen, in this case, egg-white.

The adsorption theories regarding anaphylatoxin production were materially weakened by the demonstration that a clear solution of peptone could toxify rat serum (Table 85). The evidence here presented of the action of distilled water may be said to effectually dispose of such theories, and since the reaction in this case involves a sensitized serum, it will be apparent that it has a direct bearing upon the phenomenon of anaphylaxis.

Action of Distilled Water on Normal Rat Serum.—It has been shown that distilled water exerts a remarkable action on sensitized rat serum; such being the case, it was in order to inquire as to the behavior of this reagent with normal serum. The first experiments made with this object in view are given in Table 138. For convenience in comparison, the experiments there given are designated as C and D, the dilutions corresponding to those of the same letters in Table 137.

Experiment D was made on the same day, as a control for the corresponding tests with sensitized serum of like dilution (Table 137, D); a comparison of these 2 experiments will show a striking difference. Thus, while the sensitized serum in the given dilution (9 + 1) is promptly toxified, within 5 minutes, the normal serum, under identical conditions as to dilution (18 + 2), temperature, and time, showed practically no effect. In view of the results obtained in Exper. C, it is reasonable to expect that a longer incubation of Mixture D would have resulted in poison-production.

For Exper. C, which was made 5 days later, a mixture of 36 c.c. of distilled water and 6 c.c. of fresh normal rat serum was made, each constituent being previously warmed to 37 C. A portion was tested at once (No. 1), while the balance was returned to the water bath to be retested at intervals, as indicated in the table. These tests serve as controls for those of Exper. C, Table 137, and the results deserve careful comparison. If the speed of the reaction was slow in Exper. D, it was equally so with this dilution of normal serum. In contrast with Exper. C of Table 137, where the mixture was toxified in 5 minutes, in this series the mixture failed to kill until after incubation for 45 minutes; not only the speed, but also the amount of poison produced was less, for while the 0.5 c.c. serum equivalent, in the former, was rendered toxic within 31 minutes, in the latter, the same result was reached only after incubation for 1 hour, 45 minutes. This experiment has been repeated a number of times, and it has been found possible to have a more reactive normal serum which will be toxified within 30 minutes. The individual resistance of the animals must be considered, and a better conclusion may be derived regarding the speed of reaction, if the inoculations are made in pairs, rather than as single tests. It will be shown in Table 141 that similar dilutions, kept at 45 C., may be toxified in 7 minutes.

It is desirable to state explicitly that the symptoms and findings in the fatal shocks (Nos. 4 and 8) were absolutely typical and indistinguishable from those caused by anaphylatoxin, or in specific anaphylaxis; severe dyspnea, spasms, violent convulsions, with autopsy showing permanent maximal distention of lungs, heart beating vigorously, with entire absence of clot is an ensemble that is thoroughly characteristic. The absence of clot is noteworthy, since in test No. 10 of Table 137, a very minute clot was found; this again indicates that the sensitized serum is more labile and responds with a more marked disturbance than does normal serum; the tendency toward early clotting does not mean that an excess of anaphylatoxin is present, but rather that the disturbance induced results in the simultaneous production of something (commonly called fibrin-ferment) which accelerates the coagulation change.

It will be seen from the foregoing that it is possible to produce a kill with normal rat serum by merely incubating it with distilled water. The actual amount of serum present in the fatal shocks (Nos. 4 and 8) was 1 and 0.5 c.c., respectively. It has been pointed out on page 805, that a normal rat serum, without any addition, may be rendered more or less toxic by prolonged incubation. The rate of reaction in such experiment is very slow; it is accelerated by the addition of distilled water, and is still more intensified when the serum is in the sensitized state.

The fact that normal rat serum, when diluted with distilled water and incubated, gave rise to anaphylatoxin was of such importance that it was desirable to examine more closely into the conditions involved.

TABLE 138

Action of Distilled Water on Normal Rat Serum at 37 C.

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time at 37 C.	
C	1	209	7	40″	Nil
6 + 1	2	211	"	15′	Very slight
	3	206	"	30′	Severe
	4	200	"	45′	3′10″
	5	210	3.5	60′	Moderate
	6	208	"	1 hr. 15′	Good
	7	201	"	" 30′	Severe
	8	209	"	" 45′	5′55″
D	9	208	10	5′	Very slight
9 + 1	10	195	"	15′	" "

The dose represents 1 c.c. of serum, except in Nos. 5 to 8, where it is 0.5 c.c. The time of injection was 10 seconds for 3.5 c.c.; about 40 seconds for 7 c.c.; and 1 minute for the 10 c.c. dose.

It had been previously shown, in connection with Table 44, that while a mixture of agar and rabbit serum may be apparently toxified at 50 C., in reality, the poison was probably formed before the temperature had reached this level. A similar condition was pointed out apropos of Table 116, where marked primary toxicity was developed in rabbit blood, defibrinated and kept at 55 or 60 C. In view of these observations, it was in order to test the action of distilled water on rat serum at higher temperatures.

Two experiments made with this object in mind are given in Table 139. For these tests, and those given in Table 140, 20 rats were bled, yielding 50 c.c. of pooled serum, which was kept in cracked ice. Expers. A and B were begun 5 and 9 hours, respectively, after starting to bleed. For the former, 72 c.c. of distilled water, at room temperature, were added to 12 c.c. of the serum (0 C.), ratio, 6: 1; this mixture was tested at once, and the balance was placed at 55 C. and tested at intervals of 15 minutes.

In order to insure definiteness in results, the tests were made in pairs. It will be noted that 3 acutely fatal shocks and 2 near-kills were obtained out of 12 tests. In view of the early results in Nos. 2 and 2a, it is very certain that the full amount of the poison was produced within the first 15 minutes of the experiment. The irregularities in Series A, taken as a whole, as well as in the several pairs, afford a further excellent illustration of the variation in the resistance of guinea-pigs, rather than a change or decrease in the amount of poison.

TABLE 139

Action of Distilled Water on Normal Rat Serum (6: 1) at 55 C.

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time* at 55 C. (hr.)	
A	1	189	7	—	Nil
	1a	193	"	—	"
	2	190	"	¼	Very near-kill
	2a	192	"	"	1′32″
	3	190	"	½	Slight
	3a	190	"	"	"
	4	194	"	¾	Near-kill
	4a	193	"	"	Very slight
	5	197	"	1	Slight
	5a	198	"	"	3′40″
	6	205	"	1¼	3′28″
	6a	207	"	"	Very slight
B	7	194	"	¼	Slight
	7a	200	"	"	Very slight
	8	205	"	½	" "
	8a	208	"	"	" "
	9	197	"	¾	" "
	9a	197	"	"	" "
C	10	182	"	¼	" "
	10a	185	"	"	4′30″
	11	185	"	½	5′35″
	11a	185	"	"	Very slight
	12	205	"	¾	" "
	12a	197	"	"	" "

* The injection time ranged from 17 to 43 seconds.

It might be assumed from this experiment that distilled water does produce some poison in normal rat serum, even at 55 C., but such interpretation is not correct. It requires some time for a cold mixture, such as that employed, to attain a temperature of 55 C. An actual test with a like mixture of 72 c.c. of water, at room temperature, and 12 c.c. of water, at 0 C., showed that it took 6 minutes to reach 50 degrees; after that the rise was much slower, since in 11 minutes it had only reached 53 C. It is evident, therefore, that in the experiment with serum there was abundant time, in the 5 minutes needed to reach 50 C., to develop some poison. The fact that there was no increase after 15 minutes indicated clearly that poison-production did not go on at 55 C.

To prove that this explanation was correct, Exper. B was made. In this, the same pooled serum was used as in A. The distilled water (36 c.c.) and the serum (6 c.c.) were warmed separately at 55 C. for 5 minutes, then mixed and the mixture at once returned to the water bath. The tests, made at intervals of 15 minutes, gave practically negative results, thus demonstrating that no poison was produced at 55 C.

In order to meet the possible objection that the serum used in Exper. B had been changed by being iced for a longer time than that of the former experiment, it was decided to repeat Exper. A. For this purpose, 36 c.c. of distilled water, at room temperature, were mixed with 6 c.c. of the same pooled serum, at 0 C., this mixture was then placed at 55 C., and tested with the results given in Exper. C. The time from the start of bleeding to the beginning of this experiment was 10¾ hours. It will be seen that in this check trial, 2 of 6 tests proved fatal which indicates essentially the same amount of poison-production as in Exper. A. Consequently, the foregoing conclusion must be correct.

The important fact ascertained from the preceding experiments in Table 139 was that an exposure of rat serum at 55 C. for 5 minutes was sufficient to inactivate it as to poison-production by means of distilled water. This being the case, it was next in order to make similar experiments at 50 C. Fortunately, it was possible to use for these new experiments the same pooled serum which had been employed for those in Table 139. This serum had been kept in cracked ice, over night, and for Exper. A of Table 140, it was used 23 hours after the start of bleeding.

The procedure for Exper. A was the same as for the corresponding one of the day before (Table 139). The mixture was prepared by bringing together 48 c.c. of distilled water, at room temperature, and 8 c.c. of pooled serum, at 0 C., after which it was placed at once at 50 C. The tests were made, in pairs, at intervals of 15 minutes, with results given in Table 140. The poison-producing capacity of the serum had not been injured by icing for about 23 hours. Of 8 tests, 6 proved fatal. The second pair of tests gave only slight or moderate effects, and if the experiment had been stopped at this point, it might have been supposed that the poison had decreased in amount; that such was not the case is seen from the next 4 tests which proved fatal. This merely again illustrates how the variable resistance of the guinea-pig may lead to wrong conclusions.

TABLE 140

Action of Distilled Water on Normal Rat Serum (6: 1) at 50 C.

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time* at 50 C. (hr.)	
A	1	207	7	¼	3′40″
	1a	207	“	“	4′40″
	2	195	“	½	Slight
	2a	191	“	“	Moderate
	3	195	“	¾	6′
	3a	195	“	“	6′10″
	4	194	“	1	2′50″
	4a	194	“	“	3′
B	5	195	“	¼	1′30″
	5a	195	“	“	Nil
	6	203	“	½	“
	6a	203	“	“	Very slight
	7	199	“	¾	“ “
	7a	199	“	“	“ “
C	8	185	“	¼	3′22″
	8a	190	“	“	8′ 2″
	9	186	“	½	3′
	9a	189	“	“	2′50″
	10	190	“	¾	Severe
	10a	187	“	“	2′35″

* The injection time ranged from 25 to 43 seconds.

The excellent results in Exper. A of this series, if taken by themselves, could easily be interpreted as proving that anaphylatoxin was made by incubation at 50 C. This conclusion, however, might be fallacious for reasons previously cited, so to test this point, Exper. B was made. For this, 36 c.c. of distilled water and 6 c.c. of the same pooled serum (24 hours) were warmed separately at 50 C. for 17 minutes; after which they were mixed, and the flask was returned at once to the water bath. Tests were now made at intervals of 15 minutes, with the rather surprising result shown in Table 140, B. The effects were practically nil, with the exception of test No. 5, in which death occurred as rapidly as in No. 2a of Table 139.

The marked absence of toxicity could not be explained by any change in the serum, since this experiment was made an hour after Exper. A. The only explanation seemed to be that exposure of the serum to 50 C. for 17 minutes rendered it inactive. However, as a control, Exper. C was made. Here again, the same pooled serum was used, it now being 26 hours from the start of bleeding; for the last 2 hours, the serum had been kept at room temperature. A mixture of 36 c.c. of distilled water and 6 c.c. of the serum, both at room temperature, was made, and at once placed at 50 C. When tested, as before, in pairs, it showed that the serum was just as reactive as when used for Exper. A. Consequently, the failure of Exper. B was due to a change in the matrix of the poison, caused by exposure for 17 minutes to a temperature of 50 C. Further, it is evident that the poison-production in A and C occurred probably while the temperature was rising to that level, and not after it had been reached.

The fact that the preliminary heating of normal rat serum, at 50 C. for 17 minutes, altered it so that when mixed with distilled water and incubated at 50 C. practically no poison was produced, naturally led to a similar experiment at 45 C. For this purpose, 8 rats were bled and the resulting 30 c.c. of pooled, clear serum were at once placed in cracked ice. In Exper. A, the serum was used 5 hours after the start of bleeding. The mixture was made by bringing together 36 c.c. of distilled water, at room temperature, and 6 c.c. of the serum, at 0 C.; it was at once placed at 45 C., and tests were made, in pairs, at intervals of 15 minutes. It will be seen from the results given in Table 141, that every test gave a fatal shock, thereby indicating perhaps that more poison had been produced than in the corresponding experiments at 50 and 55 C. (Tables 140, 139).

Although unlikely, it was decided to ascertain whether exposure of the serum at 45 C. would impair its capacity to produce poison. For this purpose, 36 c.c. of distilled water and 6 c.c. of pooled serum were warmed separately at 45 C. for 35 minutes, after which they were mixed and returned to the water bath. The results of these tests are given in Exper. B (Table 141); it will be seen that such treatment of the serum does not alter its reactivity. In other words, the matrix of the poison is not injured by heating at 45 C. for 35 minutes, whereas at 50 C. for 17 minutes, or at 55 C. for 5 minutes, actually renders it nonreactive to distilled water.

A striking fact brought out in connection with the experiments given in Tables 139, 140, and 141, was the possibility to toxify the serum in the 6: 1 mixture within 15 minutes. In Table 138, this result was apparently not reached until after incubation at 37 C. for 45 minutes. This fact indicated that a temperature higher than 37 C. was more favorable to poison-production, and, if so, it was reasonable to expect that at 45 C. a toxic dose could be obtained at maximal speed.

TABLE 141

Action of Distilled Water on Normal Rat Serum (6: 1) at 45 C.

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time* at 45 C.	
A	1	180	7	¼ hr.	3′58″
	1a	184	"	"	3′20″
	2	190	"	½ hr.	1′50″
	2a	196	"	"	3′15″
	3	185	"	¾ hr.	4′ 5″
	3a	190	"	"	2′55″
B	4	204	"	¼ hr.	3′10″
	4a	204	"	"	2′18″
	5	190	"	½ hr.	Very severe
	5a	190	"	"	2′20″
	6	178	"	¾ hr.	3′
	6a	172	"	"	3′ 5″
C	7	200	"	7 min.	2′25″
	7a	194	"	"	2′50″
	8	200	"	12 min.	Slight
	8a	196	"	"	Very slight
	9	198	"	17 min.	3′45″
	9a	195	"	"	3′15″

* The injection time ranged from 15 to 40 seconds.

Accordingly, Exper. C was made to test this point. For this, the same pooled serum was employed as in Expers. A and B. The distilled water (36 c.c.) and the serum (6 c.c.) were warmed separately at 45 C. for 15 minutes, and then combined, the mixture being at once returned to the water bath. The tests were planned for 5, 10, and 15 minutes, but the actual time from that of mixing until end of the injection was 7, 12, and 16 minutes. The mixture was toxified within 7 minutes, so that a dose, the serum equivalent of which was 1 c.c., was acutely fatal. Here again, as in Exper. A of Table 140, the second pair of tests failed to give anything more than mild effects, the guinea-pigs being unquestionably more resistant than the other 4. In another experiment, made with the same serum and at the same time as Exper. C, the conditions being alike, after incubation for 30 minutes, the mixture killed in a dose, the serum equivalent of which was 0.5 c.c.

TABLE 142

ACTION OF DISTILLED WATER ON NORMAL RAT SERUM (6: 1), AT 45 C. (A); UNDILUTED SERUM AT 45 C. (B)

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time* at 45 C. (min.)	
A	1	193	7	7	Very slight
	1a	196	"	"	" "
	2	193	"	11	3′
	2a	190	"	"	2′38″
	3	190	"	16	2′50″
	3a	190	"	"	2′30″
B	4	192	2	15	Slight
	5	193	"	30	"
	5a	193	"	"	"
	6	185	"	45	Very slight
	6a	185	"	"	Moderate

* The injection time for Nos. in Exper. A was 20 to 30 seconds; Exper. B, 3 to 8 seconds.

As the speed of toxification was of importance, it was decided to repeat Exper. C with another serum. Accordingly, on the following day, 6 rats were bled and yielded 21 c.c. of an opalescent, lipoidal serum. As before, 36 c.c. of distilled water and 6 c.c. of this serum were warmed separately at 45 C. for 15 minutes, then combined and the mixture returned to the water bath. It was tested at 7, 11, and 16 minutes, this time being that from moment of mixing till the end of the respective injections. The results of this experiment are given in Table 142, Exper. A, and are to be compared with those of Exper. C, in the preceding table.

While the first 2 tests were practically negative, those at 11 and 16 minutes were acutely fatal. The autopsies of Nos. 2 and 2a made, respectively 5½ and 4 minutes after death, showed the presence of very slight clot in each heart. This may be taken to mean that half the dose used would have killed without tendency to clot, as has been shown to be the case in Exper. C., Table 137. In Nos. 3 and 3a, the examination at 4½ and 3 minutes after death revealed no sign of clot, the heart beating vigorously; in all 4, the lungs were in maximal distention. The failure to toxify within 7 minutes may have been due to the fact that the serum was lipoidal; it is also possible that the guinea-pigs happened to be resistant, in the same way as Nos. 8 and 8a of Table 141.

Inasmuch as it was possible.that the excellent results obtained in these speed experiments could be due to the direct action of the heat of 45 C., it was deemed best to determine this point by suitable controls. For this purpose, 6 c.c. of the same pooled serum were placed at 45 C., and after incubation for one-fourth, one-half, and three-quarters of an hour, portions of 1 c.c. were tested, 3 pairs of tests being thus made. The effect in all 6 tests was nil.

As a further, and more conclusive, test, 10 c.c. of the same pooled serum were incubated at 45 C. and then tested in 2 c.c. dose, at the same intervals. The results of this series are given in Exper. B, Table 142. Even 2 c.c. of undiluted serum is not toxified at 45 C., in the time employed. No. 6a, however, showed a moderate reaction with some dyspnea, spasms, and was nearly thrown; this result may be interpreted as due to a very susceptible animal, but it is also possible that the serum was beginning to show some evidence of poison-production, as it was at this time incubated at 45 C. for 45 minutes. This experiment is deserving of repetition and extension.

It is indeed possible that the normal rat serum when kept at 45 C. for some time may become labilized, so that on subsequent dilution with distilled water (6: 1), at 45 C., poison may be produced almost instantaneously. With this in mind, some of the same pooled serum as used in Expers. A and B, Table 142, was placed at 45 C.; after incubating for one-fourth, one-half, and three-fourths of an hour, portions of 1 c.c. were removed, and each was diluted with 6 c.c. of distilled water. also at 45 C., and the resulting mixtures were at once injected. The actual contact of the heated serum with the distilled water, from the time of mixing until the end of injection, was but 30 to 45 seconds.

The tests which were made in pairs, at one-fourth and one-half of an hour, showed practically no effect. Of the tests made at the three-quarter hour interval, 1 proved fatal in 19 minutes, while its companion showed only slight effects. This experiment is extremely suggestive of the possibility of producing a nonspecific sensitized, or labilized serum by mere heat action.

Since it has been shown that the mere heating of normal rat serum at 50 C. induces an alteration which is recognized by the fact that such heated serum is no longer toxified by distilled water, it was of interest to see if this could be rendered toxic by means of agar. The experiments made with this objective showed that agar-gel could toxify a rat serum which had been heated at 45 and 50 C. for half an hour. The results of these trials are given in Table 55, where it will be seen that the same serum which was heated to 50 C. for half an hour was less reactive with agar than when heated at 45 C. for the same time. The former on agar treatment was not toxified in dose of 1 c.c., but with 2 c.c. it was possible to get some kills. Since distilled water is unable to toxify normal rat serum which has been heated at 50 C. (Table 140), or at 55 C. (Table 139), it follows that the suspensoid agar is considerably more active as an inducer of the reaction. This, of course, can be expected when it is recalled that agar can toxify a rat serum in ratio of 1:400,000.

It may be assumed that exposure to the temperatures mentioned inaugurates a change in the matrix which, if continued, results in a permanent modification which is either irreversible, or such that it cannot undergo the intramolecular change necessary to poison-production. A moderate exposure may partially change the matrix-complex, so that while water is unable to cause reversion, or transformation, this can be done by the more powerful agar. In other words, the labile matrix is subject to 2 types of change: First, it undergoes aggregation at a relatively low temperature, lower than that which is effective with the other serum-proteins, forming a tautomeric, inactive modification; and second, it is transformed readily into another tautomeric, physiologically active form, anaphylatoxin.

Since agar was able to toxify normal rat serum which had been heated to 50 C. for half an hour, it was desirable to see if reversion changes occurred when such heated serum was kept at 0° C. for some time. If prolonged cold exerted an action of this kind, then subsequent treatment with distilled water should give evidence of poison-production. An experiment having this purpose was accordingly made.

Nine c.c. of a fresh pooled serum were divided into 2 portions: One of these (A), 7 c.c., was placed at 50 C. for half an hour, then in cracked ice and set aside in an ice-box for 22 hours; the other portion (B), 2 c.c., was iced directly for the same time. The next day, both portions were diluted with 6 parts of distilled water and placed at 45 C. After incubation for 15 minutes, the control, or portion B, produced acute fatal shock in 2 guinea-pigs, the dose being 1 c.c. serum equivalent. By contrast, the mixture with the previously inactivated serum, portion A, likewise tested in pairs, after incubation for one-fourth, one-half, and three-fourths of an hour gave practically no effect, the dose being the same as for the controls. It is evident, therefore, that prolonged keeping at 0 C. does not cause a reversion, or restore the lability to a serum which has been inactivated by heating to 50 C. for half an hour.

To explain the results here presented from the ferment standpoint, it would be necessary to assume that the ferment is either destroyed or that an antiferment is formed when the serum is heated for a few minutes at 50 C. This view is difficult to reconcile with the mass of facts accumulated in this investigation. It seems to be more reasonable to assume that the matrix is an extremely labile body which readily undergoes intramolecular changes yielding either an inert, or a toxic modification.

Action of Horse Serum on That of Rat Sensitized to Horse Serum. —There is reason to believe, from the results presented in Table 133, that egg-white, which is totally alien to the blood stream, induces a greater change in the physical state of the plasma constituents, that is, labilizes more energetically, than any normal serum. This would seem to be, a priori, what might be expected, and the following in-vitro experiments actually appear to indicate less lability in the serum of rats sensitized with horse serum than in that with egg-white (Table 135). This, however, holds true only when the antigen is used; with distilled water, the sensitized sera behave about alike (Tables 137 and 144).

The rats employed for the following experiments were sensitized by 3 intravenous injections, 0.01, 0.5, and 3 c.c. of horse serum, respectively; the injections were given in the course of 2 months. Of these, 2 were bled 17 days after the last injection, and the pooled serum (7 c.c.) was used for Exper. A. On the 18th day, 3 more were bled, yielding 6 c.c. of pooled serum which was employed for Exper. B.; for Exper. C, 3 others were bled, also on the 18th day, and this gave

8 c.c. of pooled serum. The pools were warmed in a beaker of water at about 37 C., as also was the antigen, before making the mixtures. These were prepared according to the subjoined plan.

A. 1.4 c.c. horse serum + 7 c.c. sensitized rat serum (1:5).
B. 1.2 c.c. " " + 6 c.c. " " " (1:5).
C. 1.6 c.c. " " + 8 c.c. " " " (1:5).
D. 1.4 c.c. " " + 7 c.c. normal " " (1:5).

The mixtures were kept in the warm water, and the tests were made at the times indicated in Table 143. The time given is that from the moment of mixing until the end of injection. The dose given represents 2 c.c. of sensitized serum, except in Nos. 7 and 8, where it is 3 c.c.

TABLE 143

ACTION OF HORSE SERUM ON SENSITIZED RAT SERUM (1: 5), A, B, C, E; CONTROLS D AND F

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time at 37 C. (min.)	
A	1	205	2.4	1	Practically nil
	2	187	"	3	Near-kill
	3	188	"	15	Very severe
B	4	195	"	2	Intense quiet shock
	5	187	"	5	5 hrs. " "
	6	194	"	8	12 hrs. " "
C	7	177	3.6	1′15″	2¼ hrs. " "
	8	195	"	4	10 hrs.
D	9	196	2.4	1	Nil
	10	199	"	3	"
	11	189	"	5	"
E	12	185	4.8	1′20″	4′20″
	13	175	2.4	7	Slight
	14	184	"	15	Very slight
	15	178	"	30	" "
F	16	181	4.8	3′30″	4½ hrs. Quiet shock. Temp. in 4 hrs., 23.8 C.

In Exper. A, very severe effects followed the injection of Nos. 2 and 3. Without doubt, a dose of 4.8 c.c., such as used later (Table 135) would have killed, but the mixture was certainly not as toxic as in that experiment where 1.4 c.c. was fatal. The symptoms were those of typical anaphylactic shock: dyspnea, spasms, convulsions and the animals were thrown. Prompt recovery followed. The effect of the first injection, which was made within 1 minute, was nil.

On the other hand, the tests in Expers. B and C, although made under nearly identical conditions, presented an entirely different picture. With the exception of No. 8, there was an entire absence of violent effects, the animals reacting with an intense quiet shock; they showed prostration, became paralyzed, and the temperature dropped quickly, that of No. 5 in 4 hours being 25.4 C. Death occurred after some hours; at autopsy, the blood was found to be free from clot, a condition usually encountered where gradual asphyxiation takes place. The difference in the results of Exper. A, as contrasted with those of Expers. B and C, was probably due to variations in the temperature at which the mixtures were kept, and for that reason, in all subsequent experiments of this kind (Table 135, etc.), the Roux water bath was employed.

Experiment D served as a control and showed that the addition of horse serum to normal rat serum, under the conditions given, does not produce any poisonous effect. It would appear from the preceding experiments that the blood of rats sensitized to horse serum is not as reactive as that from such as have been treated with egg-white (Table 135). To fully establish this point, however, it was necessary to make further tests under more exact conditions, as to temperature and dosage.

For this purpose, 5 rats were sensitized by intravenous injection of 0.25 c.c. of horse serum; they were bled on the 19th day, yielding 14 c.c. of pooled serum. This, as well as some antigen, and distilled water were first warmed in a Roux water bath at 37 C., and thereupon 2 mixtures were made: 2 c.c. of horse serum + 10 c.c. of sensitized rat serum (1:5), and 0.8 c.c. of distilled water + 4 c.c. of sensitized rat serum (1:5). These were returned to the water bath and tested at times indicated in Table 143.

The control Exper. F resulted in a quiet shock, with paralysis, and drop in temperature to 23.8 C., at half an hour before death; the effects being much the same as those in Expers. B and C, including a noncoagulable blood. This test shows that the serum pool possessed a primary toxicity in the dose employed, or, 4 c.c. It might be assumed that the slow toxic action observed was due to the action of the distilled water on the sensitized serum. In view of the action of water on normal rat serum, it may be expected to have an even more rapid action on one that is sensitized, and this is actually the case, but the dilutions must be considered (Table 144). The fact that a similar test with the labile egg-white serum (Table 135, No. 6) showed no effect after incubation for 4 minutes may be taken as evidence against the assumption.

Although test No. 16 must be interpreted as showing that the pooled serum possessed a primary toxicity in dose of 4 c.c., the result of test No. 12 in Exper. E cannot mean anything else than that an increase in toxicity had occurred as a consequence of the short contact with the antigen. The serum being on the threshold, as it were, it was easy to increase the poisonous property so that an acute death occurred. The failure to produce severe effects in Nos. 13 to 15, where half the dose was used, shows that the amount of poison produced was not very great. In the corresponding experiment with egg-white (Table 135, No. 3), 1.4 c.c. of the mixture killed; this fact again indicates that the serum sensitized by means of horse serum is not as reactive to antigen as that with egg-white.

Action of Distilled Water on Serum of Rat Sensitized to Horse Serum.—Having shown that the mixture of horse serum and sensitized serum gave rise to poison-production, in vitro, though perhaps less readily than in the case of egg-white, it was desirable to demonstrate next the action of distilled water on such sensitized serum. For this purpose, 4 rats which had been sensitized by intravenous injection of 1 c.c. of horse serum 15 days before, were bled, and the pooled serum (16 c.c.) and some distilled water were warmed to 37 C. in the Roux water bath, after which 4 mixtures were made.

A. 4 c.c. of the pooled serum, with no addition, to serve as control
B. 12 c.c. distilled water + 4 c.c. pooled serum. (3 + 1)
C. 24 c.c. " " + 4 c.c. " " (6 + 1)
D. 36 c.c. " " + 4 c.c. " " (9 + 1)

These mixtures were then placed at 37 C., and tested at intervals, as indicated in Table 144.

A study of Table 144 in connection with the corresponding one with egg-white (Table 137) is of interest. It justifies the conclusion that rat serum which is sensitized to horse serum behaves with distilled water in the same way as that sensitized with egg-white. It will be seen (Exper. A) that in both sets the undiluted serum, in dose of 1 c.c., was practically without effect. Dilution B, in dose of 1 c.c. serum equivalent produced a severe shock after incubation for 30 minutes, while the corresponding test in Table 137, No. 8, gave an acute kill. This difference does not necessarily mean that the latter mixture contained more poison, since it is possible that the factor of individual variation in guinea-pigs played a part.

On the other hand, a comparison of the C dilutions might favor that of Table 144, were it not for this variable factor of animal resistance. It is to be noted that the mixture was toxic after incubation for 5 minutes, in dose of 1 c.c. serum equivalent; and likewise, toxic in half that dose, after 15 minutes. The 0.25 c.c. serum equivalent produced very severe shocks in 4 of 6 tests.

The tests with Dilution D likewise showed rapid production of poison, and it is to be specially noted that the dose of 0.25 c.c. serum equivalent caused acute death at the 1-hour period (No. 23). These results should be compared with those in Table 138, which shows the behavior of normal serum under like conditions. A comparison may also be made with Tables 141 and 142, but the mixtures in the latter were incubated at 45 C., whereas those in this series (Table 144) were kept at 37 C.

TABLE 144

Action of Distilled Water on Serum of Rat Sensitized to Horse Serum

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time at 37 C. (min.)	
A	1	180	1	5	Nil
	2	200	"	15	Very slight
	3	197	"	30	Practically nil
	4	203	"	45	Moderate
B	5	205	4	½	Nil
3 + 1	6	204	"	5	Slight
	7	196	"	15	Very slight
	8	182	"	30	Severe
C	9	206	7	1	Very slight
6 + 1	10	203	"	5	3′50″
	11	192	3.5	15	4′53″
	12	185	1.75	30	Severe shock
	13	181	"	45	Slight
	14	183	"	60	Moderate
	15	180	"	75	Very severe
	16	198	"	90	Near-kill
	17	181	"	105	Severe
D	18	210	10	1′20″	Very slight
9 + 1	19	207	"	5	2′40″
	20	200	5	15	Severe, quiet
	21	208	"	30	Very severe
	22	208	"	45	3′
	23	203	2.5	60	3′40″
	24	204	"	75	Moderate

The injection time in Exper. B was 10 to 15 seconds; the 7 c.c. dose was given in 17 to 40 seconds, while the 10 c.c., in about 1 minute. The autopsies made 3 minutes after death were perfectly typical in all but No. 19, where a very slight clot was found (bulk effect).

This series of experiments confirms and extends the results obtained with the serum of rats sensitized to egg-white. There seems to be no doubt but that the serum of a sensitized rat is considerably more labile than normal serum. It may be desirable, however, to make further tests with the latter, the mixtures being kept at 37 C., in order to make certain that the results given in Table 138 hold for other normal rat sera. The rapid toxification, even to 0.25 c.c. serum equivalent, by the mere addition of water is a fact of much importance. This, and the further fact that rat serum, on incubation with clear solution of peptone, etc., develops anaphylatoxin, indicates that a suspended solid is not necessary to this reaction. In other words, adsorption theories may find it difficult to account for the production of poison under these conditions.

TABLE 145

ACTION OF RABBIT, BEEF, AND GUINEA-PIG SERUM ON CORRESPONDING SENSITIZED RAT SERA (1: 5)

Exper.	Guinea-Pig		Mixture		Result
	No.	Weight	Amount (c.c.)	Time at 37 C.	
A	1	203	4	50″	Moderate
Rabbit	2	197	“	5′20″	Slight
B	3	195	4.8	30″	Slight
Beef	4	187	“	5′	5′
	5	178	1.9	18′	3′15″
C	6	203	4.8	35″	Moderate
Beef	7	199	“	5′20″	Nil
Control	8	205	2.4	15′	Severe
D	9	178	4.8	4′20″	3′45″
Guinea-Pig	10	177	1.2	15′	Moderate

Action of Rabbit, Beef, and Guinea-Pig Serum on Corresponding Sensitized Rat Sera.—A limited number of tests were made with rat sera sensitized by means of other antigens than those hitherto mentioned.

Only one experiment (A) was made with the object of producing an in-vitro poison by combining rabbit serum with the correspondingly sensitized rat serum. Three rats which had received an intravenous injection of 0.25 c.c. of rabbit serum 16 days before were bled, yielding 7 c.c. of pooled serum. This and the antigen were warmed at 37 C., then combined in the ratio of 1 : 5; a test was made at once, the balance being returned to the bath and retested later. The results as shown in Table 145, A, were very moderate.

A test similar to the foregoing was made with beef serum. Four rats which had been sensitized by intravenous injection of 0.25 c.c. of beef serum 16 days before, were bled, yielding 10 c.c. of pooled serum. This, together with some beef serum, was warmed at 37 C., then combined in the ratio of 1 : 5 and tested in the usual way. In view of the fact that beef serum itself may be very toxic, a control test, Exper. C, was made by adding beef serum to normal rat serum, both having been previously warmed to 37 C.

Lastly, Exper. D was made with guinea-pig serum as antigen. The rats were sensitized by intravenous injection of 0.25 c.c. of the serum. They were bled 24 days later, and the usual 1 : 5 mixture was made and tested with the result given in the table.

With regard to the rabbit serum test, Exper. A, it would appear that the serum was less active than that sensitized with horse serum. Further tests may be desirable to determine whether this difference is constant. By contrast, in Exper. B, the sensitized serum responded to its antigen, beef serum, quite promptly, since 2 deaths were obtained. The autopsies in Nos. 4, 5, and 9 were in every way typical. The severe shock in the control test No. 8 may indicate that the beef serum may have some toxifying action on normal rat serum. In Exper. D, with guinea-pig serum as antigen, a positive result was apparently obtained after incubation for 4 minutes. In the absence of a number of controls with the same pooled serum, caution must be used in accepting this as evidence of poison-production, since it has been shown in Table 121 that rat serum may be fatal in dose of 4 c.c.

NONCOAGULABILITY OF BLOOD IN SHOCKED GUINEA-PIGS

One of the effects noted in connection with anaphylactic shock is a more or less incoagulable blood. This condition is usually looked upon as a secondary phenomenon, whereas, in reality, it should be considered as a primary effect which proceeds, pari passu, with the production of poison; that is to say, noncoagulation and poisonous action are undoubtedly twin manifestations of one and the same reaction. It is not the intention, at this point, to consider the question of coagulation in general, but rather to emphasize the blood condition in shock, more especially in the guinea-pig.

It is a well known fact that dogs in anaphylactic shock yield an incoagulable blood which has been compared with that of peptone- or proteose-poisoning. On the other hand, in shock guinea-pigs, the results obtained hitherto have not been at all definite. Indeed, it is doubtful whether any one had obtained from this species a blood which showed more than a moderate degree of retardation. Delayed coagulation is found in ordinary asphyxia; it is therefore to be expected in protracted anaphylactic death. It has been frequently observed, in the course of these studies, that in guinea-pigs which died an hour or more after anaphylatoxin injection, the examination made 20 to 30 minutes, or later, after death, showed the blood in the heart to be fluid and free of the slightest trace of clot. Instances of this kind, for example, were noted in connection with Table 143.

Even in the acute specific anaphylactic shock, where death occurs in from 3 to 4 minutes, the heart when examined 12 to 15 minutes later, and even as late as 24 minutes after death (Table 146, No. 2), has been found to be free of clot. When, however, some of the blood was drawn from the exposed heart with the aid of a syringe and transferred to a small test tube, it coagulated regularly in from 4 to 6 minutes, whereas normal blood, under like conditions, clotted in half this time. This result was obtained, no matter whether the blood was drawn 2 or 20 minutes after death. For a long time all attempts to produce in the shocked guinea-pig a strictly noncoagulable blood, corresponding to that of the dog, led to no other result than a mere delay in the process.

A blood may be rendered incoagulable by contact with the pleura; this is especially true in shocked animals. The following observation may serve to illustrate this point: A guinea-pig sensitized to egg-white was given a second injection of 2 c.c. 11 days later. Immediately after the injection, the heart was exposed and the blood was drawn up into a syringe previously rinsed in distilled water; it had been planned to remove a large amount of blood and transfer it as quickly as possible to the test tube, but the fall in blood pressure was so marked and so rapid that, although the heart was entered within 1½ minutes after the end of the injection, it required nearly 3 minutes to draw up 2½ c.c. of blood. When transferred to the test tube, this blood clotted firmly within 2 minutes, the total time from the start of injection being 6½ minutes; within a minute after the blood had clotted in the tube, the heart was opened and found to be entirely free of any sign of coagulation. The opening of the heart allowed considerable blood to flow into the pleural cavity, and some of this was at once transferred by means of a syringe to a test tube. This blood remained fluid for more than 24 hours, whereas the portion which was taken direct from the heart clotted in 2 minutes.

It is well known that the circulating blood which has been rendered incoagulable by an injection, after a variable interval, returns to its previous normal state. Thus, the blood of a dog, after peptone injection, may be incoagulable within 2 minutes, and this condition may persist for an hour or more. The fact that in shocked .guinea-pigs, the blood begins to clot within the heart in about 15 minutes after the injection indicates a similar return to the normal state. The poisonous property which develops in the blood in shock, likewise does not remain intact in the blood vessels, but rapidly recedes.

It seemed reasonable, therefore, to assume that the toxic and noncoagulable properties were essentially expressions of one phase of colloidal disturbance. The enormous mass of the body might be looked upon as exerting a reversive influence, which becomes evident by the disappearance of toxicity and return of coagulability. To demonstrate, therefore, the presence of a really incoagulable phase in the shocked guinea-pig, it was necessary to modify the technic so as to insure the removal of the blood from the reversive influence of the body-mass as quickly as possible.

TABLE 146

Noncoagulability of Blood in Acute Shock in the Guinea-Pig Treated with Egg-White

Guinea-Pig		Time* of Bleed	Result
No.	Weight		
1	195	1′45″ - 2′45″	Solid clot in 2 min.
2	230	2′35″ - 3′20″	No clot for 24 hr.
3	190	3′ - 4′10″	" " " 1 hr. Then lost
4	178	3′ 8″ - 4′ 3″	" " " 7 hr.; clotted in 19 hr.

* The time is that from the end of injection during which blood was drawn into the pipet.

It was also desirable to avoid all unnecessary contact with glass, water, metal and even air bubbles. Hence, the syringe, which had been used for much of the early work in transferring blood from the heart to the test-tube, was discarded, and replaced by the clean, dry heart pipet, from which the rod was removed. By applying a little suction, after the tip was inserted into the exposed heart, it was possible to obtain 2 or 3 c.c., at maximal speed. The drawing up of air bubbles was most carefully avoided. As soon as the desired amount of blood was drawn, the pipet was sealed with vaselin.

The procedure just given was followed in the tests given in Table 146. The guinea-pigs had been sensitized to egg-white, and the second injection of 1 c.c. was given intravenously on the 13th day (Nos. 1 and 2), the 14th day (No. 3) and on the 81st day (No. 4). Shock symptoms at once became manifest. The section of Nos. 1 and 2 was begun at once after the injection, while that of Nos. 3 and 4 was delayed for 2 minutes. The time required to fully expose the heart was only 30 to 45 minutes.

It will be seen in Table 146 that the blood which was transferred within 2½ minutes after the injection clotted as rapidly as the normal one. This means, of course, that there had not been sufficient time to induce the fullest possible dislocation within the plasma. On reference to Table 132, it will be seen that the shocked blood, at about this point, is likewise unchanged as to toxicity. On the other hand, blood drawn after 2½ minutes had elapsed from the time of the injection showed perfect noncoagulability for hours. The blood of No. 3, in about 20 minutes showed a slight viscosity, but this disappeared in about 5 minutes, and the blood remained perfectly fluid, the last examination being made at the end of one hour. ·The accidental loss of the material prevented further observations. In No. 4, there was absolutely no sign of thickening or viscosity, and the fluid blood was under observation for 7 hours; the next morning, 19 hours after drawing, the contents of the tube had firmly clotted; coagulation, however, had not taken place until after the corpuscles had completely settled. The blood of No. 2 showed in 15 minutes a slight viscosity, but this soon disappeared, and the blood remained perfectly fluid for more than 24 hours, after which observations were discontinued.

These 3 positive tests conclusively show that it is possible to obtain noncoagulable blood from a shocked guinea-pig. It must be remembered that the bloods of different species, and even of the same species, may vary in their capacity to form anaphylatoxin, and also in their response as to alteration in coagulability. Differences of this kind have been touched on in Parts 5 and 7. It would be of interest to extend the series of tests given in Table 146, so as to ascertain the time at which coagulability is restored. Further, it is desirable that the same method be applied to other species in the shock state.

A number of tests were made with the noncoagulable blood of No. 2 to see if it was capable of clotting. On the supposition that a large glass surface favored clotting, a portion was drawn up into a capillary tube and freely moved back and forth, but there was no evidence of coagulation for the duration of the experiment, which was

30 minutes. As a check, it may be added that some blood was drawn up into a like capillary from the heart of No. 4, 20 minutes after the injection; this coagulated into a solid in 5 minutes. Consequently, it may be inferred that a large glass surface does not particularly aid clotting. For further tests, the noncoagulable blood of No. 2 was placed in portions of 0.5 c.c. in 4 small test tubes; the addition of one-fourth c.c. of distilled water to No. 1 caused it to clot in about 8 minutes; the addition of one-fourth c.c. of normal rat serum likewise effected coagulation in 7 minutes; the addition of one-fourth c.c. of a dilute $CaCl_2$ solution resulted in a clot in from 6 to 8 minutes; while the most rapid effect was obtained by adding a few drops of KCNS, which made a firm clot in 3 minutes.

The general conclusion to be drawn from this work is that the guinea-pig, when subjected to specific shock, responds with the production of a poisonous as well as noncoagulable state of the blood. The 2 results are temporary in character, and may be considered as a passing phase which is influenced by the mass action of the plasma, and especially that of the tissues. The timely removal from the body, thereby avoiding this mass action, stabilizes the condition so that incoagulability and toxicity persist and can be demonstrated.

SUMMARY

The rat has been found to be an invaluable reagent in the study of anaphylaxis. After previous sensitization, the test injections of corresponding antigens, such as egg-white, and diverse sera, yield, at most, a moderate respiratory disturbance. This refractory state concerning specific anaphylactic shock parallels the tolerance for multiple doses of anaphylatoxin, as well as to agar and peptone injections.

A more severe shock, and even death, follows the injection of very dilute antigen into sensitized rats, but is without effect in normal rats. The real agent in this shock is distilled water.

Nonspecific anaphylactic shock may be induced in sensitized rats by the injection of distilled water, in amounts which have no effect upon the normal rat. Salt solution in like dose can be tolerated with practically no effect.

The guinea-pig sensitized specifically is not more susceptible to distilled water than is the normal one. The relative behavior of the rat and guinea-pig to distilled water is paralleled in the relative ease with which the serum of the former, as compared with that of the latter, is toxified by agar. Normal guinea-pigs on rapid injection of very large doses of saline, and especially of distilled water, respond with shock effects.

The blood and perhaps the tissues of the sensitized rat are in a more labile state than in the normal rat.

The blood of the guinea-pig, which has been subjected to anaphylactic shock, if withdrawn at the proper time, is strictly noncoagulable as well as toxic.

A poisonous state is demonstrable in the blood of shocked guinea-pigs by the transfusion method. Even 2 c.c. of such blood, transfused at the proper time, may cause acute fatal shock in the recipient.

The results are more marked in animals sensitized to the strictly alien egg-white than in those which had been treated with horse serum. With a reaction time of about 1 minute, the blood is nontoxic, but becomes so when this reaction time is about 3 minutes. A reversion tends to occur and is indicated by the rapid recovery in the nonfatal shock.

A like poisonous state is demonstrable in the blood of sensitized rats after injection of very dilute antigen. The rat sensitized to the alien egg-white yields more poison than that treated with horse serum. The transfused blood of a desensitized rat behaves like that of normal rats.

A mixture of egg-white and sensitized rat serum, at 37 C., is toxified in 4 minutes, and may even kill in dose of 1.2 c.c. Normal serum does not show this speed of toxification.

A mixture of distilled water and sensitized rat serum is rapidly toxified at 37 C., the speed increasing with the dilution. The dose containing 1 c.c. serum equivalent, incubated for 5 minutes, is fatally toxic (Table 137).

A mixture of horse serum and sensitized rat serum can be toxified at 37 C. in from 1 to 2 minutes, so that in dose of 2 to 3 c.c. of rat serum equivalent, it produces subacute death. The larger dose of 4 c.c. causes typical acute shock.

A mixture of distilled water and serum of rat sensitized to horse serum is likewise rapidly toxified at 37 C.; it may even be fatal in dose of 0.25 c.c. serum equivalent (Table 144).

Mixtures of diverse sera with their corresponding antisera are likewise promptly toxified at 37 C.

The normal rat serum when heated at 55 for 5 minutes, or at 50 for 17 minutes, becomes inactivated and no longer will produce poison when mixed with distilled water; it may yield some on treatment with agar.

The matrix which yields the poison is therefore readily destroyed by exposure to 50 C., or higher.

When distilled water is allowed to act on normal rat serum, the maximal speed of toxification appears to occur at about 45 C.; in 7 minutes it is possible to toxify such serum so that it is acutely fatal in 1 c.c. dose (Table 141).

The speed of poison-production in normal serum or in sensitized serum is the same as that which has been demonstrated for agar, trypanosomes, peptone, etc.

The in-vivo production of anaphylatoxin in specific shock, and its production, in vitro, in mixtures of sensitized sera and antigen or distilled water, is therefore an accomplished fact. The results contraindicate the theories of adsorption and of proteolysis.

The specific anaphylactic shock is the result of anaphylatoxin production, in corpore, consequent upon the inducing action of a body which is formed by the union, or otherwise, of antigen and its specific antibody. It is not necessary for this inducing body to exist as a visible precipitate, since substances in solution are capable of giving rise to anaphylatoxin. The specificity of the reaction concerns the production of this inducing substance, and not that of the poison; and further, the antigen is in nowise the source of the anaphylatoxin which is brought into being in shock.